(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 February 2005 (10.02.2005)

PCT

(10) International Publication Number
WO 2005/012872 A2

(51) International Patent Classification[7]: G01N

(21) International Application Number: PCT/US2004/023823

(22) International Filing Date: 23 July 2004 (23.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/490,122	25 July 2003 (25.07.2003)	US
60/518,706	10 November 2003 (10.11.2003)	US
60/541,516	3 February 2004 (03.02.2004)	US
Not furnished	23 July 2004 (23.07.2004)	US

(71) Applicant *(for all designated States except US)*: **PLATYPUS TECHNOLOGIES, LLC** [US/US]; 502 South Rosa Road, Madison, WI 53719 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ISRAEL, Barbara** [US/US]; 503 Viking Road, Mt. Horeb, WI 53572 (US). **ABBOTT, Nicholas** [US/US]; 2120 Jefferson Street, Madison, WI 53711 (US). **MURPHY, Christopher** [US/US]; 1509 Wood Lane, Madison, WI 53705 (US). **HOLLISTER, Karla** [US/US]; 1618 Fordem Avenue, Apartment 317, Madison, WI 53704 (US). **SOLTANINASSAB, Syrus** [US/US]; 5305 Raymond Road, Madison, WI 53711 (US). **HANSMANN, Doug** [US/US]; 185 Acorn Lane, Libertyville, IL 60048 (US). **ACHARYA, Bharat, Raj** [US/US]; 402 North Eau Claire Avenue, Apartment 116, Madison, WI 53705 (US).

(74) Agents: **JONES, J., Mitchell** et al.; Medlen & Carroll, LLP, Suite 350, 101 Howard Street, San Francisco, CA 94105 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LIQUID CRYSTAL BASED ANALYTE DETECTION

(57) Abstract: The present invention relates to the field of detection of viruses, and in particular to detection of viruses using a liquid crystal assay format. In the present invention, virus binding in a detection region is identified by changes in liquid crystal orientation caused by virus binding independent orientation caused by any topography associated with the detection region.

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Liquid Crystal Based Analyte Detection

This application claims the benefit of U.S. Provisional Application filed 60/490,122, filed July 25, 2003; U.S. Provisional Application 60/518,706, filed November 10, 2003; and U.S. Provisional Application 60/541,516, filed February 3, 2004.

This invention was made with government support under SBIR Grant No. 5R43AI4960602 awarded by the National Institutes of Health/NIAID. The Government has certain rights in the invention.

Field of the Invention

The present invention relates to the field of detection of analytes, and in particular to detection of viruses, cells, bacteria, lipid-membrane containing organisms, proteins, nucleic acids, carbohydrates and other biomolecules, organic molecules and inorganic molecules using a liquid crystal assay format.

Background of the Invention

The detection of pathogen, protein, and nucleic acid targets in biological samples forms the basis of the multi-billion dollar *in vitro* diagnostic industry. Detection of protein and nucleic acid targets can be divided into diagnostic and research based markets. The diagnostic market includes the detection and identification of pathogens such as viruses and bacteria, the identification of various genetic markers, and the identification of markers associated with the presence of tumors. The research market includes the genomics and proteomics industries, which require analytical, drug discovery, and high-throughput screening technologies.

Initial viral diagnostics consisted of the crude, albeit sensitive and non-specific techniques of direct inoculation of sample material into suckling mice, embryonated eggs, or living cells. Diagnostic methods have since evolved to the sensitive, specific, but time consuming serological techniques of neutralization, ELISA and fluorescent antibody assays and subsequently to the current highly sensitive, instrumentation-dependent techniques of nucleic acid amplification and luminescent bead-based assays. This evolution in approach to virus detection and identification has been driven by advances in biology (cell culture, immunology), followed by advances in biochemistry (immunochemistry, molecular biology, dye chemistry). More recent progress comes from advances in instrumentation

limited to the detection of any particular change in the mesogens forming the liquid crystal. Indeed, a variety of changes may be detected, including, but not limited to a change in color, a change in texture, a change in tilt, and homeotropic orientation.

The present invention is not limited to the detection of any particular type of virus. Indeed, the detection of a variety of viruses is contemplated, including, but not limited to viruses in the following families: Adenoviridae, Arenaviridae, Astroviridae, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Iridoviridae, Filoviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Poxviridae, Reoviridae, Retroviridae, Rhabdoviridae, Togaviridae, Badnavirus, Bromoviridae, Comoviridae, Geminiviridae, Partitiviridae, Potyviridae, Sequiviridae, and Tombusviridae. In some embodiments, the virus is a Japanese Encephalitis Virus group virus. In other embodiments, the Japanese Encephalitis Virus group virus is selected from the group consisting of West Nile Virus and St. Louis Encephalitis Virus. In still further embodiments, the virus is an enveloped virus.

The present invention is not limited to the use of any particular substrate. Indeed, the use of a variety of substrates is contemplated, including, but not limited to metal films, glass, silicon, diamond and polymeric materials. The present invention is not limited to the use of any particular polymeric materials. Indeed, the use of a variety of polymeric materials is contemplated, including, but not limited to those selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate. The present invention is not limited to the use of any particular mesogen. Indeed, the use of a variety of mesogens is contemplated, including, but not limited to those selected from the group consisting of 4-cyano-4'-pentylbiphenyl, N-(4-methoxybenzylidene)-4-butlyaniline and combinations thereof. The present invention is not limited to the use of any particular virus recognition moiety. Indeed, the use of a variety of virus recognition moieties is contemplated, including, but not limited to antigen binding proteins and nucleic acids. In some embodiments, the antigen binding protein is an immunoglobulin.

In some embodiments, the substrate comprises a plurality of detection regions. In some embodiments, the plurality of detection regions have the same virus recognition moiety bound thereto. In other embodiments, the plurality of detection regions have different virus recognition moieties bound thereto. In some embodiments, the detection

bound thereto. In some embodiments, the detection device further comprises a second substrate arranged opposite said first substrate to form a cell. In still further embodiments, the plurality of detection regions are arranged in an array. In some embodiments, the substrates further comprise at least one control region comprising immobilized virus. In other embodiments, the device comprises a second substrate oriented opposite said first substrate to form a cell for containing mesogens. In still other embodiments, the devices comprise cross polar lenses oriented on either side of said first substrate and said substrate. In some embodiments, the substrate comprises microchannels in said first substrate, wherein said microchannels deliver sample to said detection region.

In still further embodiments, the present invention provides a kit comprising: a) a device for the detection of a virus comprising a first substrate comprising at least one detection region having a first virus recognition moiety specific for said virus immobilized thereon, wherein said detection region does not homeotropically orient an added mesogen in the absence of said virus; and b) instructions for detection of said virus. In some embodiments, the kit further comprises a vial containing mesogens. In other embodiments, the kit further comprises a vial containing a virus for use as a positive control.

In still further embodiments, the present invention provides methods comprising: a) providing a functionalized detection substrate treated to align mesogens, a stamp substrate displaying at least one ligand, a biological test sample suspected of containing a binding partner for the ligand, and mesogens; b) contacting the test sample with the stamp substrate under conditions such that the binding partner can bind the ligand; c) contacting the detection substrate with the stamp substrate under conditions such that the binding partner to the ligand is transferred to the detection substrate; d) detecting the presence of the binding partner to the ligand on the detection substrate by applying the mesogens to the substrate. The present invention is not limited to use with any particular biological sample. Indeed, the use of a variety of biological samples is contemplated, including, but not limited to, those selected from the group consisting of whole blood, serum, cerebral spinal fluid, nasopharyngeal aspirate, and nasal secretions. In some embodiments, the alignment of the mesogens by the detection substrate is disrupted by the presence of the binding partner to the ligand. In some preferred embodiments, the alignment is homeotropic. In further embodiments, the mesogens are not homeotropically aligned over areas of the detection substrate wherein the binding partner of the ligand is present. In still other embodiments, the detection substrate is used to form an optical cell. In some embodiments, the detecting

substrate comprises obliquely deposited gold. The present invention is not limited to the use of any particular type of ligand. Indeed, the use of a variety of ligands is contemplated. In some embodiments, the ligand is an antigenic substance from a pathogenic organism. In some embodiments, the antigenic substance is a protein. In further embodiments, the protein is an envelope protein of a virus. In some preferred embodiments, the envelope protein is protein E from West Nile Virus. In some embodiments, the kits further comprise a container containing a control binding partner. The present invention is not limited to any particular binding partner. Indeed, a variety of binding partners are contemplated. In some embodiments, the binding partner is a ligand. In some embodiments, the kits further comprise an additional substrate for forming an optical cell. In still other embodiments, the kits comprise polarized lenses.

In still further embodiments, the present invention provides systems for detecting an analyte comprising: a) a first substrate displaying a recognition moiety, wherein the recognition moiety interacts with the analyte; b) a second substrate comprising a surface configured to receive the analyte interacting with the recognition moiety; and c) a liquid crystal overlaying the second substrate. In some embodiments, the first substrate is selected from the group consisting of a stamp, a bead, and column media. In some embodiments, the stamp comprises PDMS. In some embodiments, the bead is a magnetic bead. In some embodiments, the column is immunoaffinity column media. In some embodiments, the recognition moiety is selected from the group consisting of a protein, polypeptide, peptide, nucleic acid, carbohydrate, lipid, organic molecule and inorganic molecule. In some embodiments, the liquid crystal comprises mesogens selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline). In some embodiments, the second substrate comprises a functionalized surface. In some embodiments, the functionalized surface comprises a polyimide. In some embodiments, the polyimide is rubbed. In some embodiments, the polyimide is selected from the group consisting of Nissan 7210, Nissan 3510, Nissan 410, Nissan 3140, Nissan 5291, and Japan Synthetic Rubber JALS 146-R19. In some embodiments, the polyimide homeotropically orients the liquid crystal. In some embodiments, the polyimide is selected from the group consisting of Nissan 7511L and SE 1211.

In further embodiments, the present invention provides methods of detecting an analyte comprising: a) providing a first substrate displaying a recognition moiety, a second

methods further comprise the step of contacting the analyte-recognition moiety complex with a secondary binding agent. In some embodiments, the secondary binding agent is selected from the group consisting of an antigen binding protein, and enzyme, avidin, and biotin. In some embodiments, the presence of the secondary binding agent enhances the detection of the analyte after transfer to the second substrate. In some embodiments, the secondary binding agent is complexed with a lipid. In some embodiments, the secondary binding agent is displayed on a liposome.

In still further embodiments, the present invention provides kits comprising a) a first substrate displaying a recognition moiety, wherein the recognition moiety interacts with an analyte; b) a second substrate comprising a surface configured to receive the analyte interacting with the recognition moiety; c) a vial containing mesogens; and d) instructions for detecting the analyte. In some embodiments, the first substrate is selected from the group consisting of a stamp, a bead, and column media. In some embodiments, the stamp comprises PDMS. In some embodiments, the bead is a magnetic bead. In some embodiments, the column is an immunoaffinity column. In some embodiments, the recognition moiety is selected from the group consisting of a protein, polypeptide, peptide, nucleic acid, carbohydrate, lipid, organic molecule and inorganic molecule. In some embodiments, the mesogens are selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline). In some embodiments, the second substrate comprises a functionalized surface. In some embodiments, the functionalized surface comprises a polyimide. In some embodiments, the polyimide is rubbed. In some embodiments, the polyimide is selected from the group consisting of Nissan 7210, Nissan 3510, Nissan 410, Nissan 3140, Nissan 5291, and Japan Synthetic Rubber JALS 146-R19. In some embodiments, the polyimide homeotropically orients the liquid crystal. In some embodiments, the polyimide is selected from the group consisting of Nissan 7511L and SE 1211. In some embodiments, the kits further comprise a second vial comprising a secondary binding agent. In some embodiments, the secondary binding agent is selected from the group consisting of an antigen binding protein, an enzyme, avidin and biotin.

In still further embodiments, the present invention provides methods for detecting analytes comprising: a) providing: i) a sample suspected of containing of an analyte; ii) a detection device comprising a substrate comprising at least one electrode and at least one

measuring the impedance of the detection device, wherein a change in capacitance is indicative of analyte binding. In some embodiments, the impedance is capacitance or resistance. In some embodiments, the measuring is in real-time.

In still further embodiments, the present invention provides devices for detecting an analyte comprising a first substrate comprising at least one electrode and at least one detection region, wherein the at least one electrode is configured to provide an electrical potential to attract an analyte to the substrate and to determine the presence of the analyte by measuring electrical properties of the device, and a second substrate oriented opposite of the first substrate, wherein the first substrate and the second substrate form a chamber for containing a liquid crystal. In some embodiments, the electrical property is impedance. In some embodiments, the impedance is capacitance or resistance. In some embodiments, the impedance is capacitance. In some embodiments, the substrate is selected from the group consisting of metal films, glass, silicon, diamond and polymeric materials. In some embodiments, the polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate. In some embodiments, the devices further comprise mesogens, wherein the mesogens are selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline) and combinations thereof. In some embodiments, the detection region comprises a recognition moiety. In some embodiments, the recognition moiety is selected from the group consisting of a peptide, polypeptide, protein, nucleic acid, carbohydrate, organic molecule, and inorganic molecule. In some embodiments, the protein is an antigen binding protein. In some embodiments, the first substrate comprises a plurality of detection regions. In some embodiments, the plurality of detection regions display the same recognition moiety. In some embodiments, the plurality of detection regions display different recognition moieties. In some embodiments, the at least one electrode is selected from the group consisting of interdigitated, hyperbolic, triangular and rectangular electrodes. In some embodiments, the first substrate comprises at least two electrodes.

In still further embodiments, the present invention provides systems for detection of an analyte comprising the detection device described above and a readout device, the readout device comprising an opening configured to receive the detection device and an electrical circuit that contacts the at least one electrode when the detection device is in

of visual detection, optical detection, spectroscopy, light transmission, and electrical detection.

In some embodiments, the present invention provides methods of detecting binding interaction between a ligand and its binding partner comprising: a) providing a ligand and a binding partner, wherein at least one of the ligand molecule and the binding partner molecule are complexed with a lipid, and mesogens; b) contacting the ligand molecule and the binding partner molecule under conditions such that the ligand molecule and the binding partner molecule interact to form a ligand-binding partner complex; and c) detecting the ligand-binding partner complex by contacting the complex with mesogens. In some embodiments, the mesogens are homeotropically oriented by the complex. In some embodiments, the binding partner is recognition moiety. In some embodiments, the ligand is an analyte a sample. In some embodiments, the detecting step further comprises contacting the complex to a substrate prior to contacting with the mesogens. In some embodiments, the homeotropic alignment of the mesogens is detected by a method selected from the group consisting of visual detection, optical detection, spectroscopy, light transmission, and electrical detection. In some embodiments, the analyte is selected from the group consisting of a protein, peptide, polypeptide, nucleic acid, organic molecule, inorganic molecule, virus, bacteria, liposome, cell, and fungus. In some embodiments, the substrate is selected from the group consisting of metal films, glass, silicon, diamond and polymeric materials. In some embodiments, the polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate. In some embodiments, the mesogen is selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline) and combinations thereof. In some embodiments, the substrate comprises a detection region comprising a recognition moiety. In some embodiments, the recognition moiety is selected from the group consisting of a peptide, polypeptide, protein, nucleic acid, carbohydrate, organic molecule, and inorganic molecule. In some embodiments, the protein is an antigen binding protein. In some embodiments, the substrate comprises a plurality of detection regions. In some embodiments, the plurality of detection regions display the same recognition moiety. In some embodiments, the plurality of detection regions display different recognition moieties. In some embodiments, the ligand id biotin and the

Figure 5 is an image of an assay conducted with an assay device of the present invention.

Figure 6 shows the results from a simulation of hyperbolic electrodes.

Figure 7 shows a schematic of an device configured for dielectrophoresis.

Figure 8 is an image of an assay using a polyimide coated substrate to non-specifically detect an analyte.

Figure 9 is an image of an assay using a polyimide coated substrate to non-specifically detect an analyte.

Figure 10 is a graphic representation of luminosity index for the experiment depicted in Figure 9.

Figure 11 is an image of an assay using a polyimide coated substrate to non-specifically detect an analyte.

Figure 12 is an image of the results of the detection of F1 in chicken serum.

Figure 13 is an image of the results of the same experiment as Figure 12 taken with a polarized microscope.

Figure 14 is a schematic depiction of a readout device of the present invention.

Figure 15 presents images of experiments in which labeled liposomes are used to report ligand binding.

Definitions

As used herein, the term "recognition moiety" refers to a composition of matter that interacts with an analyte of interest in either a covalent or noncovalent manner.

As used herein, the term "virus recognition moiety" refers to any composition of matter that binds specifically to a virus. Examples of "virus recognition moieties" include, but are not limited to antigen binding proteins and nucleic acid aptamers.

The term "substrate" refers to a composition that serves as a base for another composition such as recognition moiety. Examples of substrates include, but are not limited to, silicon surfaces, glass surfaces, glass beads, magnetic beads, agarose beads, etc.

As used herein, the term "ligand" refers to any molecule that binds to or can be bound by another molecule. A ligand is any ion, molecule, molecular group, or other substance that binds to another entity to form a larger complex. Examples of ligands include, but are not limited to, peptides, carbohydrates, nucleic acids, antibodies, or any molecules that bind to receptors.

As used herein, the term "homeotropic director" refers to a topographical feature

As used herein, the term "column media" refers to media used to fill a chromatography column, such as cationic exchange media, anionic exchange media, and immunoaffinity column media.

As used herein, the term "detection region" refers to a discreet area on substrate that is designated for detection of an analyte (e.g., a virus of interest) in a sample.

As used herein, the term "immobilization" refers to the attachment or entrapment, either chemically or otherwise, of a material to another entity (e.g., a solid support) in a manner that restricts the movement of the material.

As used herein, the terms "material" and "materials" refer to, in their broadest sense, any composition of matter.

As used herein the term "antigen binding protein" refers to a glycoprotein evoked in an animal by an immunogen (antigen) and to proteins derived from such glycoprotein (*e.g.*, single chain antibodies and F(ab')2, Fab' and Fab fragments). An antibody demonstrates specificity to the immunogen, or, more specifically, to one or more epitopes contained in the immunogen. Native antibody comprises at least two light polypeptide chains and at least two heavy polypeptide chains. Each of the heavy and light polypeptide chains contains at the amino terminal portion of the polypeptide chain a variable region (*i.e.*, VH and VL respectively), which contains a binding domain that interacts with antigen. Each of the heavy and light polypeptide chains also comprises a constant region of the polypeptide chains (generally the carboxy terminal portion) which may mediate the binding of the immunoglobulin to host tissues or factors influencing various cells of the immune system, some phagocytic cells and the first component (C1q) of the classical complement system. The constant region of the light chains is referred to as the "CL region," and the constant region of the heavy chain is referred to as the "CH region." The constant region of the heavy chain comprises a CH1 region, a CH2 region, and a CH3 region. A portion of the heavy chain between the CH1 and CH2 regions is referred to as the hinge region (*i.e.*, the "H region"). The constant region of the heavy chain of the cell surface form of an antibody further comprises a spacer-transmembranal region (M1) and a cytoplasmic region (M2) of the membrane carboxy terminus. The secreted form of an antibody generally lacks the M1 and M2 regions.

As used herein, the term "selective binding" refers to the binding of one material to another in a manner dependent upon the presence of a particular molecular structure (*i.e.*, specific binding). For example, an immunoglobulin will selectively bind an antigen that contains the chemical structures complementary to the ligand binding site(s) of the

can refer to surfactant molecules that associate to form surfactant molecular assemblies. The term "self-assembling monomers" includes single molecules (*e.g.*, a single lipid molecule) and small molecular assemblies (*e.g.*, polymerized lipids), whereby the individual small molecular assemblies can be further aggregated (*e.g.*, assembled and polymerized) into larger molecular assemblies.

As used herein, the term "linker" or "spacer molecule" refers to material that links one entity to another. In one sense, a molecule or molecular group can be a linker that is covalent attached two or more other molecules (*e.g.*, linking a ligand to a self-assembling monomer).

As used herein, the term "bond" refers to the linkage between atoms in molecules and between ions and molecules in crystals. The term "single bond" refers to a bond with two electrons occupying the bonding orbital. Single bonds between atoms in molecular notations are represented by a single line drawn between two atoms (*e.g.*, C-C). The term "double bond" refers to a bond that shares two electron pairs. Double bonds are stronger than single bonds and are more reactive. The term "triple bond" refers to the sharing of three electron pairs. As used herein, the term "ene-yne" refers to alternating double and triple bonds. As used herein the terms "amine bond," "thiol bond," and "aldehyde bond" refer to any bond formed between an amine group (*i.e.*, a chemical group derived from ammonia by replacement of one or more of its hydrogen atoms by hydrocarbon groups), a thiol group (*i.e.*, sulfur analogs of alcohols), and an aldehyde group (*i.e.*, the chemical group -CHO joined directly onto another carbon atom), respectively, and another atom or molecule.

As used herein, the term "covalent bond" refers to the linkage of two atoms by the sharing of two electrons, one contributed by each of the atoms.

As used herein, the term "spectrum" refers to the distribution of light energies arranged in order of wavelength.

As used the term "visible spectrum" refers to light radiation that contains wavelengths from approximately 360 nm to approximately 800 nm.

As used herein, the term "substrate" refers to a solid object or surface upon which another material is layered or attached. Solid supports include, but are not limited to, glass, metals, gels, and filter paper, among others.

As used herein, the terms "array" and "patterned array" refer to an arrangement of elements (*i.e.*, entities) into a material or device. For example, combining several types of ligand binding molecules (*e.g.*, antibodies or nucleic acids) into an analyte-detecting device,

crystals show a strong optical activity that is much higher than can be explained on the bases of the rotatory power of the individual mesogens. When light equal in wavelength to the pitch of the director impinges on the liquid crystal, the director acts like a diffraction grating, reflecting most and sometimes all of the light incident on it. If white light is incident on such a material, only one color of light is reflected and it is circularly polarized. This phenomenon is known as selective reflection and is responsible for the iridescent colors produced by chiral nematic crystals.

"Smectic," as used herein refers to liquid crystals which are distinguished from "nematics" by the presence of a greater degree of positional order in addition to orientational order; the molecules spend more time in planes and layers than they do between these planes and layers. "Polar smectic" layers occur when the mesogens have permanent dipole moments. In the smectic A2 phase, for example, successive layers show anti ferroelectric order, with the direction of the permanent dipole alternating from layer to layer. If the molecule contains a permanent dipole moment transverse to the long molecular axis, then the chiral smectic phase is ferroelectric. A device utilizing this phase can be intrinsically bistable.

"Frustrated phases," as used herein, refers to another class of phases formed by chiral molecules. These phases are not chiral, however, twist is introduced into the phase by an array of grain boundaries. A cubic lattice of defects (where the director is not defined) exist in a complicated, orientationally ordered twisted structure. The distance between these defects is hundreds of nanometers, so these phases reflect light just as crystals reflect x-rays.

"Discotic phases" are formed from molecules that are disc shaped rather than elongated. Usually these molecules have aromatic cores and six lateral substituents. If the molecules are chiral or a chiral dopant is added to a discotic liquid crystal, a chiral nematic discotic phase can form.

Description of the Invention

The present invention relates to the field of detection of analytes, and in particular to detection of viruses, cells, bacteria, lipid-membrane containing organisms, proteins, nucleic acids, carbohydrates and other biomolecules, organic molecules and inorganic molecules using a liquid crystal assay format. Liquid crystal-based assay systems (LC assays) are described in U.S. Pat. No. 6,284,197; WO 01/61357; WO 01/61325; WO 99/63329; Gupta *et al.*, Science 279:2077-2080 (1998); Seung-Ryeol Kim, Rahul R. Shah, and Nicholas L.

In addition to entities with lipids membranes, which are listed below, the devices, systems and methods of the present invention are useful for detecting a variety of analytes, including, but not limited to, the following analytes: biomolecules including polypeptides (e.g., proteins), toxins, polynucleotides (e.g., RNA and DNA), carbohydrates, viruses, mycoplasmas, fungi, bacteria, and protozoa, especially Class A agents such as Variola major (smallpox), Bacillus anthracis (anthrax), Yersinia pestis (plague), Clostridium botulinum (botulism), Francisella tularensis (tularemia), Arenaviruses (Arenaviridae), Ebola hemorrhagic fever virus, Marburg hemorrhagic fever, Lassa fever virus, Junin and related viruses (Argentinian hemorrhagic fever virus, Bolivian hemorrhagic fever virus, Brazilian hemorrhagic fever virus, Venezuelan hemorrhagic fever virus), Dengue hemorrhagic fever virus, and toxins such as botulinum and Trichothecene (T2) mycotoxins; Class B agents such as Coxiella burnetti (Q fever), Brucella sp. (brucellosis), Burkholderia mallei (glanders), Salmonella sp., Shigella dysenteria, Escherichia coli strain O 157:H7, Cryptosporidium parvum, Alphaviruses (Togaviridae family) such as Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, and toxins such as ricin toxin, epsilin toxin from Clostridium perfigens, and Staphylococcus enterotoxin B; and Class C agents such as mutlidrug resistant tuberculosis, Nipah virus, Hantaviruses, Tick-borne hemorrhagic fever viruses, Tick-borne encephalitis viruses, and Yellow fever virus.

Other analytes include, but are not limited to, acids, bases, organic ions, inorganic ions, pharmaceuticals, herbicides, pesticides, chemical warfare agents, and noxious gases. These agents can be present as components in mixtures of structurally unrelated compounds, racemic mixtures of stereoisomers, non-racemic mixtures of stereoisomers, mixtures of diastereomers, mixtures of positional isomers or as pure compounds. The detection of these analytes, and specific substrates and recognition moieties for such detection, is described in more detail in co-pending applications 10/227,974, 10/443,419, and 60/585,275; all of which are incorporated herein by reference in their entirety.

Accordingly, the present invention provides improved substrates and devices for the detection of analytes. For convenience, the description of the present invention is divided into the following sections: I. Recognition Moieties; II. Substrates; III. Functionalization of Substrates; IV. Mesogens; V. Direct Detection of Entities with Lipid Membranes; VI. Non-specific Detection Following Specific Capture; VII. Detection with Lipid Tags VIII. Kits.

hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*).

For preparation of monoclonal antibodies, it is contemplated that any technique that provides for the production of antibody molecules by continuous cell lines in culture will find use with the present invention (*See e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, NY). These include but are not limited to the hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, Nature 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (*See e.g.,* Kozbor *et al.*, Immunol. Tod., 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al., in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

In addition, it is contemplated that techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; herein incorporated by reference) will find use in producing specific single chain antibodies that serve as recognition moieties. Furthermore, it is contemplated that any technique suitable for producing antibody fragments will find use in generating antibody fragments that are useful recognition moieties. For example, such fragments include but are not limited to: F(ab')2 fragment that can be produced by pepsin digestion of the antibody molecule; Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and Fab fragments that can be generated by treating the antibody molecule with papain and a reducing agent. In still further embodiments, the recognition moiety comprises a phage displaying an antigen binding protein.

In some embodiments where the recognition moiety is a polynucleotide or polypeptide, a plurality of recognition moieties are arrayed on the substrates using photo activated chemistry, microcontact printing, and ink-jet printing. In particularly preferred embodiments, photolithography is utilized (*See e.g.*, U.S. Patent Nos. 6,045,996; 5,925,525; and 5,858,659; each of which is herein incorporated by reference). Using a series of photolithographic masks to define substrate exposure sites, followed by specific chemical synthesis steps, the process constructs high-density arrays of oligonucleotides, with each probe in a predefined position in the array. Multiple probe arrays are synthesized simultaneously on, for example, a large glass wafer. The wafers are then diced, and individual probe arrays are packaged in injection-molded plastic cartridges, which protect them from the environment and serve as chambers for hybridization.

In other embodiments, nucleic acid recognition moieties are electronically captured

In another preferred embodiment, the recognition moiety is a drug moiety. The drug moieties can be agents already accepted for clinical use or they can be drugs whose use is experimental, or whose activity or mechanism of action is under investigation. The drug moieties can have a proven action in a given disease state or can be only hypothesized to show desirable action in a given disease state. In a preferred embodiment, the drug moieties are compounds that are being screened for their ability to interact with an analyte of choice. As such, drug moieties that are useful in practicing the instant invention include drugs from a broad range of drug classes having a variety of pharmacological activities.

Classes of useful agents include, for example, non-steroidal anti-inflammatory drugs (NSAIDS). The MAIDS can, for example, be selected from the following categories: *(e.g.,* propionic acid derivatives, acetic acid derivatives, fenamic acid derivatives, biphenylcarboxylic acid derivatives and oxicams); steroidal anti-inflammatory drugs including hydrocortisone and the like; antihistaminic drugs *(e.g.,* chlorpheniranune, triprolidine); antitussive drugs *(e.g.,* dextromethorphan, codeine, carmiphen and carbetapentane); antipruritic drugs *(e.g.,* methidilizine and trimeprizine); anticholinergic drugs *(e.g.,* scopolamine, atropine, homatropine, levodopa); anti-emetic and antinauseant drugs *(e.g.,* cyclizine, meclizine, chlorpromazine, buclizine); anorexic drugs *(e.g.,* benzphetamine, phentermine, chlorphentermine, fenfluramine); central stimulant drugs *(e.g.,* amphetamine, methamphetamine, dextroamphetamine and methylphenidate); antiarrhythmic drugs *(e.g.,* propanolol, procainamide, disopyraminde, quinidine, encainide); P-adrenergic blocker drugs *(e.g.,* metoprolol, acebutolol, betaxolol, labetalol and timolol); cardiotonic drugs *(e.g.,* milrinone, amrinone and dobutamine); antihypertensive drugs *(e.g.,* enalapril, clonidine, hydralazine, minoxidil, guanadrel, guanethidine);diuretic drugs *(e.g.,* amiloride and hydrochlorothiazide); vasodilator drugs *(e.g.,* diltazem, amiodarone, isosuprine, nylidrin, tolazoline and verapamil); vasoconstrictor drugs *(e.g.,* dihydroergotamine, ergotamine and methylsergide); antiulcer drugs *(e.g.,* ranitidine and cimetidine); anesthetic drugs *(e.g.,* lidocaine, bupivacaine, chlorprocaine, dibucaine); antidepressant drugs *(e.g.,* imipramine, desipramine, amitryptiline, nortryptiline); tranquilizer and sedative drugs *(e.g.,* chlordiazepoxide, benacytyzine, benzquinamide, flurazapam, hydroxyzine, loxapine and promazine); antipsychotic drugs *(e.g.,* chlorprothixene, fluphenazine, haloperidol, molindone, thioridazine and trifluoperazine); antimicrobial drugs (antibacterial, antifungal, antiprotozoal and antiviral drugs).

Antimicrobial drugs which are preferred for incorporation into the present composition include, for example, pharmaceutically acceptable salts of β-lactam drugs,

ketorolac, are also of use. Other drugs of use in conjunction with the present invention will be apparent to those of skill in the art.

When the recognition moiety is a chelating agent, crown ether or cyclodextrin, host-guest chemistry will dominate the interaction between the recognition moiety and the analyte. The use of host-guest chemistry allows a great degree of recognition-moiety-analyte specificity to be engineered into a device of the invention. The use of these compounds to bind to specific compounds is well known to those of skill in the art. *See,* for example, Pitt *et al.* "The Design of Chelating Agents for the Treatment of Iron Overload," In, INORGANIC CHEMISTRY IN BIOLOGY AND MEDICINE; Martell, A.E., Ed.; American Chemical Society, Washington, D.C., 1980, pp. 279-312; Lindoy, L.F., THE CHEMISTRY OF MACROCYCLIC LIGAND COMPLEXES; Cambridge University Press, Cambridge,1989; Dugas, H., BIOORGANIC CHEMISTRY; Springer-Verlag, New York, 1989, and references contained therein.

Additionally, a manifold of routes allowing the attachment of chelating agents, crown ethers and cyclodextrins to other molecules is available to those of skill in the art. *See,* for example, Meares *et al.,* "Properties of In Vivo Chelate-Tagged Proteins and Polypeptides." In, MODIFICATION OF PROTEINS: FOOD, NUTRITIONAL, AND PHARMACOLOGICAL ASPECTS;" Feeney, R.E., Whitaker, 1.R., Eds., American Chemical Society, Washington, D.C., 1982, pp.370-387; Kasina *et al. Bioconjugate Chem.* 9:108-117 (1998); Song *et al., Bioconjugate Chem.* 8:249-255 (1997).

In a presently preferred embodiment, the recognition moiety is a polyaminocarboxylate chelating agent such as ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetic acid (DTPA). These recognition moieties can be attached to any amine-terminated component of a SAM or a spacer arm, for example, by utilizing the commercially available dianhydride (Aldrich Chemical Co., Milwaukee, WI).

In still further preferred embodiments, the recognition moiety is a biomolecule such as a protein, nucleic acid, peptide or an antibody. Biomolecules useful in practicing the present invention can be derived from any source. The biomolecules can be isolated from natural sources or can be produced by synthetic methods. Proteins can be natural proteins or mutated proteins. Mutations can be effected by chemical mutagenesis, site-directed mutagenesis or other means of inducing mutations known to those of skill in the art. Proteins useful in practicing the instant invention include, for example, enzymes, antigens, antibodies and receptors. Antibodies can be either polyclonal or monoclonal. Peptides and

be unmasked following the synthesis, at which time it becomes available for reaction with a SAM component or a spacer arm.

In other preferred embodiments, the peptide is attached directly to the substrate (*See*, Frey *et al. Anal. Chem. 68*:3187-3193 (1996)). In a particularly preferred embodiment, the peptide is attached to a gold substrate through a sulfhydryl group on a cysteine residue. In another preferred embodiment, the peptide is attached through a thiol to a spacer arm which terminates in, for example, an iodoacetamide, chloroacetamide, benzyl iodide, benzyl bromide, alkyl iodide or alkyl bromide. Similar immobilization techniques are known to those of skill in the art (*See,* for example, Zull *et al. J. Ind Microbiol.* 13:137-143 (1994)).

In another preferred embodiment, the recognition moiety forms an inclusion complex with the analyte of interest. In a particularly preferred embodiment, the recognition moiety is a cyclodextrin or modified cyclodextrin. Cyclodextrins are a group of cyclic oligosaccharides produced by numerous microorganisms. Cyclodextrins have a ring structure which has a basket-like shape. This shape allows cyclodextrins to include many kinds of molecules into their internal cavity (*See,* for example, Szejtli, J., CYCLODEXTRINS AND THEIR INCLUSION COMPLEXES; Akademiai Klado, Budapest, 1982; and Bender *et al.,* CYCLODEXTRIN CHEMISTRY, Springer-Verlag, Berlin, 1978).

Cyclodextrins are able to form inclusion complexes with an array of organic molecules including, for example, drugs, pesticides, herbicides and agents of war (*See,* Tenjarla *et al., J. Pharm. Sci.* 87:425-429 (1998); Zughul *et al., Pharm. Dev. Technol.* 3:43-53 (1998); and Albers *et al., Crit. Rev. Ther. Drug Carrier Syst.* 12:311-337 (1995)). Importantly, cyclodextrins are able to discriminate between enantiomers of compounds in their inclusion complexes. Thus, in one preferred embodiment, the invention provides for the detection of a particular enantiomer in a mixture of enantiomers (*See,* Koppenhoefer *et al. J. Chromatogr. A* 793:153-164 (1998)).

The cyclodextrin recognition moiety can be attached to a SAM component, through a spacer arm or directly to the substrate (*See,* Yamamoto *et al., J. Phys. Chem. B* 101:6855-6860 (1997)). Methods to attach cyclodextrins to other molecules are well known to those of skill in the chromatographic and pharmaceutical arts (*See,* Sreenivasan, *Appl. Polym. Sci.* 60:2245-2249 (1996)).

In other embodiments, the recognition moieties can be nucleic acids (e.g., RNA or DNA) or receptors that are specific for a particular entity (e.g., virus). In some

antigenic molecule from a pathogenic organism (such as those listed above). In preferred embodiments, the antigenic molecule (e.g., a protein) is recognized by an antibody molecule in the body fluid of a test subject that has been exposed to the pathogenic organism. In particularly preferred embodiments, the ligand is protein E from the envelope of West Nile Virus.

In some preferred embodiments, the ligands or recognition moieties are complexed with a lipid. The present invention contemplates complexation of the recognition moiety with a variety of lipids and lipid containing materials, including, but not limited to, fatty acids, phospholipids, mono-, di- and tri-glycerides comprising fatty acids and/or phospholipids, lipid bilayers, and liposomes. The lipid containing material can be provided as multilayers, as well as braided, lamellar, helical, tubular, and fiber-like shapes, and combinations thereof. Standard attachment chemistries are available for attaching a recognition moiety or ligand of interest to lipids and lipids containing materials. These attachment chemistries are described in more detail below with reference to liposomes.

In some preferred embodiments, the present invention utilizes liposomes. A variety of methods are useful for producing liposomes. Such methods are described in detail in numerous articles and have been reviewed in texts such as New (New, Liposomes: A Practical Approach, IRL Press, Oxford, [1989]), and Rosoff (Rosoff, Vesicles, Marcel Dekker, Inc., New York, [1996]) among others. See also, U.S. Pat. Nos. 6,183,772, 6,306,598, 6,180,784, 6,740,643, and 6,706,922, all of which are incorporated herein by reference, for methods of forming liposomes and other lipid containing materials. In some preferred embodiments, the liposomes are prepared using a probe sonication methods. Methods of derivatizing lipids with a diverse range of compounds (e.g., carbohydrates, proteins, nucleic acids, and other chemical groups) are well known in the art. The carboxylic acid on the terminal end of lipids can be easily modified to form esters, phosphate esters, amino groups, ammoniums, hydrazines, polyethylene oxides, amides, and many other compounds. These chemical groups provide linking groups for carbohydrates, proteins, nucleic acids, and other chemical groups (e.g., carboxylic acids can be directly linked to proteins by making the activated ester, followed by reaction to free amine groups on a protein to form an amide linkage). Examples of antibodies attached to Langmuir films are known in the art (See e.g., Tronin et al., Langmuir 11: 385 [1995]; and Vikhohn et al., Langmuir 12: 3276 [1996]). There are numerous other means to couple materials to membranes, or incorporate materials within a membrane, including for example, coupling of proteins or nucleic acids to polymer membranes (See e.g., Bamford et al. Adv. Mat. 6:

standard techniques (See, *e.g.*, Goodman, C.H.L., Crystal Growth Theory and Techniques, Plenum Press, New York 1974). Alternatively, the crystals can be purchased commercially *(e.g.,* Fischer Scientific). The crystals can be the sole component of the substrate or they can be coated with one or more additional substrate components. Thus, it is within the scope of the present invention to utilize crystals coated with, for example one or more metal films or a metal film and an organic polymer. Additionally, a crystal can constitute a portion of a substrate which contacts another portion of the substrate made of a different material, or a different physical form *(e.g.,* a glass) of the same material. Other useful substrate configurations utilizing inorganic crystals and/or glasses will be apparent to those of skill in the art.

B. Inorganic oxides

In other embodiments of the present invention, inorganic oxides are utilized as the substrate. Inorganic oxides of use in the present invention include, for example, Cs_20, $Mg(OH)_2$, $Ti0_2$, $Zr0_2$, $Ce0_2$, Y_20_3, Cr_20_3, Fe_20_3, NiO, ZnO, $A1_20_3$, $Si0_2$ (glass), quartz, In_20_3, $Sn0_2$, $Pb0_2$ and the like. The inorganic oxides can be utilized in a variety of physical forms such as films, supported powders, glasses, crystals and the like. A substrate can consist of a single inorganic oxide or a composite of more than one inorganic oxide. For example, a composite of inorganic oxides can have a layered structure (*i.e.*, a second oxide deposited on a first oxide) or two or more oxides can be arranged in a contiguous non-layered structure. In addition, one or more oxides can be admixed as particles of various sizes and deposited on a support such as a glass or metal sheet. Further, a layer of one or more inorganic oxides can be intercalated between two other substrate layers (*e.g.*, metal-oxide-metal, metal-oxide-crystal).

In a presently preferred embodiment, the substrate is a rigid structure that is impermeable to liquids and gases. In this embodiment, the substrate consists of a glass plate onto which a metal, such as gold is layered by evaporative deposition. In a still further preferred embodiment, the substrate is a glass plate ($Si0_2$) onto which a first metal layer such as titanium has been layered. A layer of a second metal such as gold is then layered on top of the first metal layer.

C. Metals

In still further embodiments of the present invention, metals are utilized as substrates. The metal can be used as a crystal, a sheet or a powder. The metal can be

In a presently preferred embodiment, the substrate is permeable and it consists of a layer of gold, or gold over titanium, which is deposited on a polymeric membrane, or other material, that is permeable to liquids, vapors and/or gases. The liquids and gases can be pure compounds *(e.g.,* chloroform, carbon monoxide) or they can be compounds which are dispersed in other molecules *(e.g.,* aqueous protein solutions, herbicides in air, alcoholic solutions of small organic molecules). Useful permeable membranes include, but are not limited to, flexible cellulosic materials *(e.g.,* regenerated cellulose dialysis membranes), rigid cellulosic materials *(e.g.,* cellulose ester dialysis membranes), rigid polyvinylidene fluoride membranes, polydimethylsiloxane and track etched polycarbonate membranes.

In a further preferred embodiment, the layer of gold on the permeable membrane is itself permeable. In a still further preferred embodiment, the permeable gold layer has a thickness of about 70 Angstroms or less.

In those embodiments wherein the permeability of the substrate is not a concern and a layer of a metal film is used, the film can be as thick as is necessary for a particular application. For example, if the film is used as an electrode, the film can be thicker than in an embodiment in which it is necessary for the film to be transparent or semi-transparent to light.

Thus, in a preferred embodiment, the film is of a thickness of from about 0.01 nanometer to about 1 micrometer. In a further preferred embodiment, the film is of a thickness of from about 5 nanometers to about 100 nanometers. In yet a further preferred embodiment, the film is of a thickness of from about 10 nanometers to about 50 nanometers.

E. Formats

The substrates of the present invention are provided in a variety of formats. For examples, the substrates may present planar or curved surfaces or be beads. The bead format is especially useful for the indirect detection methods described below. The bead substrates of the present invention may comprise any of the substrate materials described above. In some preferred embodiments, the beads are commercially available beads such as agarose beads, acrylic beads, or latex beads. In some embodiments, the beads are magnetic. In still other embodiments, the beads are coated with a metal such as silver or gold. In still other embodiments, substrates such column chromatography media may be used to capture analytes. Examples of such substrates include immunoaffinity columns (i.e., columns containing media functionalized with antigen binding proteins), protein-A affinity columns, cation exchange columns such as S-SEPHAROSE, SP-SEPHAROSE, and carboxymethyl

embodiments, the SAM is formed from $CH_3(CH_2)_{15}SH$ and $CH_3(CH_2)_4SH$ or $CH_3(CH_2)_{15}SH$ and $CH_3(CH_2)_9SH$. In any of the above described embodiments, the carbon chains can be functionalized at the ω-terminus (*e.g.*, NH_2, COOH, OH, CN), at internal positions of the chain (*e.g.*, aza, oxa, thia) or at both the ω-terminus and internal positions of the chain.

A recognition moiety can be attached to the surface of a SAM by any of a large number of art-known attachment methods. In one preferred embodiment, a reactive SAM component is attached to the substrate and the recognition moiety is subsequently bound to the SAM component via the reactive group on the component and a group of complementary reactivity on the recognition moiety (*See, e.g.*, Hegner *et al. Biophys. J.* 70:2052-2066 (1996)). In another preferred embodiment, the recognition moiety is attached to the SAM component prior to immobilizing the SAM component on the substrate surface: the recognition moiety-SAM component cassette is then attached to the substrate. In a still further preferred embodiment, the recognition moiety is attached to the substrate via a displacement reaction. In this embodiment, the SAM is preformed and then a fraction of the SAM components are displaced by a recognition moiety or a SAM component bearing a virus recognition moiety. In still other embodiments, the polypeptide recognition moieties are adsorbed directly onto hydrophobic monolayers such as $CH_3(CH_2)_{15}SH$. In embodiments where the recognition moiety is an antibody or other molecule that binds to protein A, protein A is first attached to the monolayer followed by the antibody, which is bound by protein A.

B. Functionalized SAMs

The discussion which follows focuses on the attachment of a reactive SAM component to the substrate surface. This focus is for convenience only and one of skill in the art will understand that the discussion is equally applicable to embodiments in which the SAM component-recognition moiety is preformed prior to its attachment to the substrate. As used herein, "reactive SAM components" refers to components that have a functional group available for reaction with a recognition moiety or other species following the attachment of the component to the substrate.

Currently favored classes of reactions available with reactive SAM components are those that proceed under relatively mild conditions. These include, but are not limited to nucleophilic substitutions (*e.g.*, reactions of amines and alcohols with acyl halides), electrophilic substitutions (*e.g.*, enamine reactions) and additions to carbon-carbon and

b. 7-oct-1-enyl trichlorosilane 8-hydroxyoctyl

2. Diol (dihydroxyalkyl) siloxanes (silylate surface and hydrolyze to diol)
   a. (glycidyl trimethoxysilane (2,3-dihydroxypropyloxy)propyl
3. Aminoalkyl siloxanes (amines requiring no intermediate functionalizing step).
   a. 3-aminopropyl trimethoxysilane aminopropyl
4. Dimeric secondary aminoalkyl siloxanes
   a. bis (3-trimethoxysilylpropyl) amine bis(silyloxylpropyl)amine.

It will be apparent to those of skill in the art that an array of similarly useful functionalizing chemistries are available when SAM components other than siloxanes are used. Thus, for example similarly functionalized alkyl thiols can be attached to metal films and subsequently reacted to produce the functional groups such as those exemplified above.

In another preferred embodiment, the substrate is at least partially a metal film, such as a gold film, and the reactive group is tethered to the metal surface by an agent displaying avidity for that surface. In a presently preferred embodiment, the substrate is at least partially a gold film and the group which reacts with the metal surface comprises a thiol, sulfide or disulfide such as:

$$Y\text{-}S\text{-}R^2\text{-}X^2 \qquad (2)$$

R^2 is a linking group between sulfur and X^2 and X^2 is a reactive group or a protected reactive group. X^2 can also be a recognition moiety as discussed below. Y is a member selected from the group consisting of H, R^3 and R^3-S-, wherein R^2 and R^3 are independently selected. When R^2 and R^3 are the same, symmetrical sulfides and disulfides result, and when they are different, asymmetrical sulfides and disulfides result.

A large number of functionalized thiols, sulfides and disulfides are commercially available (Aldrich Chemical Co., St. Louis). Additionally, those of skill in the art have available to them a manifold of synthetic routes with which to produce additional such molecules. For example, amine-functionalized thiols can be produced from the corresponding halo-amines, halo-carboxylic acids, *etc.* by reaction of these halo precursors with sodium sulfhydride. *See, e.g.*, Reid, ORGANIC CHEMISTRY of BIVALENT SULFUR, VOL 1, pp. 21-29, 32-35, vol. 5, pp. 27-34, Chemical Publishing Co., New York, 1.958, 1963. Additionally, functionalized sulfides can be prepared via alkylthio-de-halogenation with a mercaptan salt (*See,* Reid, ORGANIC CHEMISTRY OF

such as, for example, nitrobenzyl derivatives, phenacyl groups, benzoin esters, etc. Other such cleaveable groups are well-known to those of skill in the art.

In another preferred embodiment, the organosulfur compound is partially or entirely halogenated. An example of compounds useful in this embodiment include:

$$X^1Q_2C(CQ^1{}_2)_mZ^1(CQ^2{}_2)_nSH \qquad (5)$$

wherein, X^1 is a member selected from the group consisting of H, halogen reactive groups and protected reactive groups. Reactive groups can also be recognition moieties as discussed below. Q, Q^1 and Q^2 are independently members selected from the group consisting of H and halogen. Z^1 is a member selected from the group consisting of $-CQ_2-$, $-CQ^1{}_2-$, $-CQ^2{}_2-$, -O-, -S-, NR^4-, $-C(O)NR^4$ and $R^4NC(O0-$, in which R^4 is a member selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl and heterocyclic groups and m and n are independently a number between 0 and 40.

In yet another preferred embodiment, the organic layer comprises a compound according to Formula 5 above, in which Q, Q^1 and Q^2 are independently members selected from the group consisting of H and fluorine. In a still further preferred embodiment, the organic layer comprises compounds having a structure according to Formulae (6) and (7):

$$CF_3(CF_2)_mZ^1\ (CH_2)_nSH \qquad (6)$$

$$CF_3(CF_2)_oZ^2(CH_2)_PSH \qquad (7)$$

wherein, Z^1 and Z^2 are members independently selected from the group consisting of $-CH_2-$, -O-, -S-, NR^4 , $-C(O)NR^4$ and $R^4NC(O)-$ in which R^4 is a member selected from the group consisting of H, alkyl, substituted alkyl, aryl, substituted aryl, heteroaryl and heterocyclic groups. In a presently preferred embodiment, the Z groups of adjacent molecules participate in either an attractive (*e.g.*, hydrogen bonding) or repulsive (*e.g.*, van der Waals) interaction.

In Formula 7, m is a number between 0 and 40, n is a number between 0 and 40, o is a number between 0 and 40 and p is a number between 0 and 40.

In a further preferred embodiment, the compounds of Formulae 6 and 7 are used in conjunction with an organosulfur compound, either halogentated or unhalogenated, that bears a recognition moiety.

al., PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

In a preferred embodiment, the SAM component bearing the recognition moiety is attached directly and essentially irreversibly via a "stable bond" to the surface of the substrate. A "stable bond", as used herein, is a bond which maintains its chemical integrity over a wide range of conditions *(e.g.,* amide, carbamate, carbon-carbon, ether, *etc.*). In another preferred embodiment, the SAM component bearing the recognition moiety is attached to the substrate surface by a "cleaveable bond". A "cleaveable bond", as used herein, is a bond that is designed to undergo scission under conditions which do not degrade other bonds in the recognition moiety-analyte complex. Cleaveable bonds include, but are not limited to, disulfide, imine, carbonate and ester bonds.

In certain embodiments, it is advantageous to have the recognition moiety attached to a SAM component having a structure that is different than that of the constituents of the bulk SAM. In this embodiment, the group to which the recognition moiety is bound is referred to as a "spacer arm" or "spacer." Using such spacer arms, the properties of the SAM adjacent to the recognition moiety can be controlled. Properties that are usefully controlled include, for example, hydrophobicity, hydrophilicity, surface-activity and the distance of the recognition moiety from the plane of the substrate and/or the SAM. For example, in a SAM composed of alkanethiols, the recognition moiety can be attached to the substrate or the surface of the SAM via an amine terminated poly(ethyleneglycol). Numerous other combinations of spacer arms and SAMs are accessible to those of skill in the art.

The hydrophilicity of the substrate surface can be enhanced by reaction with polar molecules such as amine-, hydroxyl- and polyhydroxylcontaining molecules. Representative examples include, but are not limited to, polylysine, polyethyleneimine, poly(ethyleneglycol) and poly(propyleneglycol). Suitable functionalization chemistries and strategies for these compounds are known in the art (*See,* for example, Dunn, R.L., *et al.*, Eds. POLYMERIC DRUGS AND DRUG DELIVERY SYSTEMS, ACS Symposium Series Vol. 469, American Chemical Society, Washington, D.C. 1991).

The hydrophobicity of the substrate surface can be modulated by using a hydrophobic spacer arm such as, for example, long chain diamines, long chain thiols, α, ω-amino acids, *etc.* Representative hydrophobic spacers include, but are not limited to, 1,6-hexanediamine, 1,8-octanediamine, 6-aminohexanoic acid and 8-aminooctanoic acid.

substrate's surface by "underlabeling" the surface functional groups with less than a stoichiometric equivalent of the first component. The first component can be a SAM component liked to a terminal reactive group or recognition group, a spacer arm or a monovalent moiety. Subsequently, the second component is contacted with the substrate. This second component can either be added in stoichiometric equivalence, stoichiometric excess or can again be used to underlabel to leave sites open for a third component.

C. Polyimides

In some embodiments, the substrates are coated with polyimide layer. It is contemplated that polyimide coated substrates are especially useful because in some instances, the surfaces homeotropically orient a liquid crystal, while in other instances the surfaces can be rubbed to provide an anisotropic surface for orient a liquid crystal. In preferred embodiments, a substrate such as a silicon wafer is coated with a polyimide. In preferred embodiment, the substrate is spin coated with the polyimide. A variety of polyimides find use with the present invention, including, but not limited to Nissan 7210, Nissan 3510, Nissan 410, Nissan 3140, Nissan 5291, and Japan Synthetic Rubber JALS 146-R19 for planar alignment of liquid crystals and Nissan 7511L and SE 1211 for homeotropic orientation of liquid crystals. Surprising, it has been found that the ability of rubbed polyimide surfaces to orient liquid crystals is maintained when a recognition moiety is displayed on the rubbed surface, and then masked when an analyte binds the recognition moiety. Thus, areas where an analyte is bound have a non-ordered liquid crystal and appear white or bright when viewed through cross polars and areas where analyte is not bound remain ordered and appear dark when viewed through cross polars. Surprising, it has also been found that polyimide surfaces that homeotropically orient liquid crystals can be used to report non-specific binding to the surface. In these embodiments, areas where an analyte is bound have a disordered liquid crystal appear white or bright when viewed through cross polars and areas where no analyte is bound maintain the homeotropic orientation and appear dark. These different polyimides provide different anchoring properties and different binding affinity to different proteins which can be used to probe and report the binding events between the proteins. Likewise, different liquid crystals show different response to the specific binding event. Therefore, it is possible to tune the assays by using different liquid crystalline materials such as, 5CB, BL093, TL 216, ZLI 5800, MLC 6613, and (p-methoxybenzylidene)-p-butylaniline (MBBA) with different optical and dielectric properties.

The translation stage moves along each of the rows of the array and the appropriate reagent is delivered to each of the reaction site. For example, the A amidite is delivered only to the sites where amidite A is to be coupled during that synthesis step and so on. Common reagents and washes are delivered by flooding the entire surface and then removing them by spinning.

In still further embodiments, virus recognition moieties are spotted onto a suitable substrate. Such spotting can be done by hand with a capillary tube or micropipette, or by an automated spotting apparatus such as those available from Affymetrix and Gilson (*See e.g.*, U.S. Pat. Nos. 5,601,980; 6,242,266; 6,040,193; and 5,700,637; each of which is incorporated herein by reference).

E. Blocking

In some embodiments, following immobilization of the recognition moiety on the surface of the substrate, the remainder of the substrate is blocked to guard against non-specific binding to the substrate surface. Examples of suitable blocking agents, include, but are not limited to, serum albumins, zwitterionic polymers, adsorbed lipid layers, dextran and other sugars, cross-linked lipids, polyethylene oxide, polyoxazolines, hydrogels, and milk. In preferred embodiments, the blocking agent bovine serum albumin, human serum albumin or equine serum albumin.

IV. Mesogens

Any compound or mixture of compounds which forms a mesogenic layer can be used in conjunction with the present invention. The mesogens can form thermotropic or lyotropic liquid crystals. Both the thermotropic and lyotropic liquid crystals can exist in a number of forms including nematic, chiral nematic, smectic, polar smectic, chiral smectic, frustrated phases and discotic phases.

The mesogenic layer can be a substantially pure compound, or it can contain other compounds which enhance or alter characteristics of the mesogen. Thus, in one preferred embodiment, the mesogenic layer further comprises a second compound, for example and alkane, which expands the temperature range over which the nematic and isotropic phases exist. Use of devices having mesogenic layers of this composition allows for detection of the analyte recognition moiety interaction over a greater temperature range.

In some preferred embodiments, the mesogenic layer further comprises a dichroic dye or fluorescent compound. Examples of dichroic dyes and fluorescent compounds useful in the present invention include, but are not limited to, azobenzene, BTBP, polyazo compounds, anthraquinone, perylene dyes, and the like. In particularly preferred embodiments, a dichroic dye of fluorescent compound is selected that complements the orientation dependence of the liquid crystal so that polarized light is not required to read the assay. In some preferred embodiments, if the absorbance of the liquid crystal is in the visible range, then changes in orientation can be observed using ambient light without crossed polars. In other preferred embodiments, the dichroic dye or fluorescent compound is used in combination with a fluorimeter and the changes in fluorescence are used to detect changes in orientation of the liquid crystal.

V. Direct Detection of Entities With Lipid Membranes

The present invention provides methods and devices for the direct detection of entities having a biological membrane, including viruses and bacteria that are pathogens. The systems and devices of the present invention can be of any configuration that allows for the contact of a mesogenic layer with an organic layer or inorganic layer (*e.g.*, metal, metal salt or metal oxide). The only limitations on size and shape are those that arise from the situation in which the device is used or the purpose for which it is intended. The device can be planar or non-planar. Thus, it is within the scope of the present invention to use any number of polarizers, lenses, filters lights, and the like to practice the present invention.

The systems and devices of the present invention find use in the detection of variety of viruses and entities having lipid membranes. Examples of such entities having lipid membranes include, but are not limited to, viruses, bacteria, liposomes, cells, mycoplasmas, protozoans, fungi and the like.

The present invention is not limited to the detection of any particular type of virus. Indeed, the present invention contemplates the detection of a variety of viruses, including viruses from the following families: Adenoviridae, Arenaviridae, Astroviridae,

human herpesvirus 5, human cytomegalovirus, mouse cytomegalovirus 1, human herpesvirus 6, human herpesvirus 4, ateline herpesvirus 2, frog virus 3, flounder virus, goldfish virus 1, influenza A virus, influenza B virus, influenza C virus, Thogoto virus, murine polyomavirus, cottontail rabbit papillomavirus (Shope), Paramyxovirus, human parainfluenza virus 1, measles virus, mumps virus, human respiratory syncytial virus, mice minute virus, B19 virus, adeno-associated virus 2, poliovirus 1, human rhinovirus 1A, porcine rhinovirus, hepatitis A virus, encephalomyocarditis virus, St. Louis encephalomyocarditis virus, foot-and-mouth disease virus O, vaccinia virus, orf virus, fowlpox virus, sheeppox virus, monkey pox virus, myxoma virus, swinepox virus, Molluscum contagiosum virus, Yaba monkey tumor virus, reovirus 3, bluetongue virus 1, simian rotavirus SA11, Colorado tick fever virus, golden shiner virus, mouse mammary tumor virus, murine leukemia virus, avian leukosis virus, Mason-Pfizer monkey virus, bovine leukemia virus, human immunodeficiency virus 1, human spumavirus, vesicular stomatitis Indiana virus, rabies virus, bovine ephemeral fever virus, Sindbis virus, rubella virus, commelina yellow mottle virus, alfalfa mosaic virus, tobacco streak virus, brome mosaic virus, cucumber mosaic virus, tomato spotted wilt virus, apple stem grooving virus, carnation latent virus, cauliflower mosaic virus, beet yellows virus, cowpea mosaic virus, broad bean wilt virus 1, tobacco ringspot virus, carnation ringspot virus, pea enation mosaic virus, soil-borne wheat mosaic virus, maize streak virus, beet curly top virus, bean golden mosaic virus, barley stripe mosaic virus, raspberry bushy dwarf virus, barley yellow dwarf virus, maize chlorotic mottle virus, maize rayado fino virus, tobacco necrosis virus, white clover cryptic virus 1, white clover cryptic virus 2, potato virus X, potato virus Y, ryegrass mosaic virus, barley yellow mosaic virus, Fiji disease virus, wound tumor virus, rice ragged stunt virus, potato yellow dwarf virus, tobacco necrosis satellite, parsnip yellow fleck virus, rice tungro spherical virus, Southern bean mosaic virus, rice stripe virus, tobacco mosaic virus, tobacco rattle virus, carnation mottle virus, tomato bushy stunt virus, apple chlorotic leaf spot virus, turnip yellow mosaic virus, carrot mottle virus.

The present invention is not limited to the detection of any particular type of bacteria. Indeed, the detection of variety of bacteria is contemplated, including, but not limited to Gram-positive cocci such as Staphylococcus aureus, Streptococcus pyogenes (group A), Streptococcus spp. (viridans group), Streptococcus agalactiae (group B), S. bovis, Streptococcus (anaerobic species), Streptococcus pneumoniae, and Enterococcus spp.; Gram-negative cocci such as Neisseria gonorrhoeae, Neisseria meningitidis, and Branhamella catarrhalis; Gram-positive bacilli such as Bacillus anthracis, Bacillus subtilis,

particularly in immunocompromised patients such as those with AIDS. Preferred organisms include Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus, Saccharomyces cerevisiae, Aspergillus fumigatus, and Aspergillus nidulans. See Goodman and Gilman's Pharmacological Basis of Therapeutics, (8th ed., 1990) Table 44-1, page 1024-1033, for additional microbial pathogens, diseases, and current therapeutic agents. The above-described cells are generally available, for example, from the American Type Culture Collection.

The present invention is not limited to the detection of any particular types of cells. Examples of such cells include, but are not limited to, Chinese hamster ovary cells (CHO-K1, ATCC CCl-61); bovine mammary epithelial cells (ATCC CRL 10274; bovine mammary epithelial cells); monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; see, *e.g.*, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; rat fibroblasts (208F cells); MDBK cells (bovine kidney cells); human hepatoma line (Hep G2), and, for example, the following cancerous cells or cells isolated from the following carcinomas: fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, Ewing's tumor, lymphangioendotheliosarcoma, synovioma, mesothelioma, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilns' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma,

mesogenic layer to provide for visual detection. Alternatively, the light can be passed through the mesogenic layer and the amount of light transmitted, absorbed or reflected can be measured. The device can utilize a backlighting device such as that described in U.S. Pat. No. 5,739,879. Light in the ultraviolet and infrared regions is also of use in the present invention. Microscopic techniques can utilize simple light microscopy, confocal microscopy, polarized light microscopy, atomic force microscopy (Hu et al., Langmuir 13:5114-5119 (1997)), scanning tunneling microscopy (Evoy et al., J. Vac. Sci. Technol A 15:1438-1441, Part 2 (1997)), and the like. Spectroscopic techniques of use in practicing the present invention include, for example, infrared spectroscopy (Zhao et al., Langmuir 13:2359-2362 (1997)), raman spectroscopy (Zhu et al., Chem. Phys. Lett. 265:334-340 (1997)), X-ray photoelectron spectroscopy (Jiang et al., Bioelectroch. Bioener. 42:15-23 (1997)) and the like. Visible and ultraviolet spectroscopies are also of use in the present invention. Other useful techniques include, for example, surface plasmon resonance (Evans et al., J. Phys. Chem. B 101:2143-2148 (1997), ellipsometry (Harke et al., Thin Solid Films 285:412-416 (1996)), electrical methods (such as impedometric methods (Rickert et al., Biosens. Bioelectron. 11:757:768 (1996)), and the like.

In some embodiments, the devices of the present invention further comprise an electrode or series of electrodes. In some preferred embodiments, at least two electrodes are provided in a plane on one of the surfaces of the device substrate. A variety of electrodes may be utilized, including, but not limited to, interdigitated, hyperbolic, triangular and rectangular electrodes. In some particularly preferred embodiments, the device comprises interdigitated electrodes. Figures 3a and 3b provide a schematic depiction of a device and preferred electrodes of the present invention. Figure 3a depicts liquid crystal molecules supported on a nanostructured surface coated with a recognition moiety (in this embodiment an antibody). In the absence of bound analyte, the mesogens assume a planar orientation. Upon binding of an analyte (in this embodiment, virus particles) on to the surface the molecules align perpendicular to the surface inducing a change in the capacitance between two electrodes. Figure 3b present a schematic of interdigitated electrodes. In this embodiment, the size of the arrows is on the order of 500 μm. Figure 7 presents a schematic depiction of a substrate configured for dielectrophoresis. The electrode is formed on the surface of the substrate by methods known in the art (e.g., photolithography, printing, etc.). The electrode includes a circuit that interfaces with power source (e.g., an alternating current source) and a phase inverter. In some embodiments, a mask (e.g., formed from PDMS) is used to contain the sample on the substrate during dielectrophoresis.

the dielectric properties of herpes simplex virus type 1 virions with dielectrophoresis. Biochimica et Biophysica Acta 1571: 1-8 (2002). Using vaccinia virus labeled with lipophilic carbocyanin dyes and nucleophilic Hoechst dyes, Akin et al. Real-time virus trapping and fluorescent imaging in microfluidic devices, Nano Letters 4: 257-259 (2003) have demonstrated real-time imaging of the capture and trapping of virus particles by dielectrophoretic filters within a microfluidic biochip. In a step towards the development of a rapid diagnostic for food-borne pathogens, Suehiro et al. Selective detection of specific bacteria using dielectrophoretic impedance measurement method combined with an antigen-antibody reaction, Journal of Electrostatics 58: 229-246 (2003) combined measurement of DEP impedance with antibody agglutination to detect bacteria in suspension.

Forces arising from DEP can be used to rapidly concentrate, manipulate, and even separate viruses from small sample volumes. The experiments described above, however, were conducted using model systems of very high concentration, purified virus (up to 10^{12} pfu/mL) suspended in media of very low ionic strength. For practical application to viral diagnostics, DEP must be utilized under conditions of physiological ionic strength ($600mSm^{-1}$ or greater) and must effectively.

The methods of the present invention contemplate dielectrophoretic forces on viruses to be of the order of 1pN. This force, when acting on a virus, generates velocities of $\sim 100\ \mu ms^{-1}$. Thus, in preferred embodiments, the time taken for the particle to travel a 100 μm distance is on the order of 1s. In contrast, Brownian forces acting on virus particles give rise of diffusion coefficients of $\sim 10^{-12}\ m^2s^{-1}$. Thus the time taken by the virus particle to diffuse through the same distance of 100 μm in absence of dielectrophoretic force is 1.4 hrs. It is thus contemplated that in preferred embodiments, dielectrophoretic forces can accelerate the transport of viruses to surfaces by 3 orders of magnitude.

In further preferred embodiments, the present of analyte in a sample is determined by measuring the dielectric capacitance of the device. The present invention is not limited to a particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present invention. Nevertheless, it is contemplated that liquid crystals have large, anisotropic electrical properties that are reflected in changes in electrical capacitance related to orientation within an electrical field. The method of the present invention, based on dielectric transduction, relies on the principle of change in capacitance between two electrodes when dielectric properties of the medium between them changes. Thus, in some embodiments of the present invention, DEP is utilized to force an analyte

The devices of the present invention can be used to detect the presence of wide variety of biological entities in a sample, including, but not limited to those described above. Likewise, the devices of the present invention can be used to detect biological entities in a variety of samples. In some embodiments, the biological sample is a biological fluid, tissue homogenate, feces, vesicular fluid, swab of an orifice or tissue, or media in which virus has been cultured or prepared. In some embodiments, the biological fluid is cerebral-spinal fluid, urine, serum, plasma, nasal secretion, sputum, semen or saliva.

Biological samples may be collected by a variety of techniques. In some embodiments, whole blood is collected by one of many routes (e.g., venipuncture or fingerstick) into a tube containing an anticoagulant such as heparin or sodium citrate. The blood is mixed and then a sample is removed and placed into contact with a sensing surface. In some embodiments, serum is obtained by permitting blood collected as described to form a clot in the tube. The tube is subjected to centrifugation or is permitted to sit for one or more hours so that the serum component separates from the cellular component. A sample of the serum is placed in contact with the sensing surface. In some embodiments, tissue homogenates are utilized. Pieces of organs (e.g., kidney, spleen, heart, brain, liver, lymph nodes) are either minced by scissors or blades or are placed into a container with fluid (PBS, other buffers, media, water, etc) and homogenized using a plastic pestle or by insertion of a mechanical homogenizer into the container until there are no large pieces of tissue visible. The preparation is centrifuged at low speed (<20,000 rpm for 5-60 minutes) to remove the particulate material remaining. The supernatant is placed in contact with the device substrate surface. In some embodiments, spinal fluid is collected from the spinal cord by a needle. The fluid is inserted into a sterile tube. A sample of the spinal fluid is placed into contact with the device substrate surface. In some embodiments, a sample of nasal secretions is collected onto a cotton or synthetic applicator swab and the swab is placed into a fluid (PBS, water, media, other buffers etc). An aliquot of the sample is placed in contact with the device substrate surface. In some embodiments, a nasopharyngeal aspirate sample is collected by insertion of the swab into the nasopharynx. The swab is placed into a tube containing fluid (PBS, media, water, buffers) and a sample of the fluid is placed in contact with the device substrate surface. In some embodiments, the biological sample is obtained from an intermediate host animal (e.g., a mosquito in the case of West Nile Virus). One or more than one mosquito is suspended in liquid such as phosphate buffered saline or other buffers or media used to grow cells in culture or water. The mosquitoes are homogenized

recognition moiety is detected by a change in the color and texture of the liquid crystal. The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the invention. Nevertheless, it is believed that the change in color and texture is due tilting of the mesogens in the liquid crystal prior to assumption of a homeotropic orientation.

Accordingly, in those embodiments utilizing light in the visible region of the spectrum, the light can be used to simply illuminate details of the mesogenic layer. Alternatively, the light can be passed through the mesogenic layer and the amount of light transmitted, absorbed or reflected can be measured. The device can utilize a backlighting device such as that described in U.S. Pat. No. 5,739,879, incorporated herein by reference. Light in the ultraviolet and infrared regions is also of use in the present invention.

In some embodiments, the cell is placed in between cross polar lenses and light is passed though the lenses and the cell. Areas of homeotropic orientation appear black, while areas of planar orientation appear bright. Thus, the presence of bound virus is indicated by a black field while areas where no virus is bound are indicated by a bright field.

In some embodiments, the present invention utilizes plate readers to detect changes in the orientation of mesogens upon binding of an analyte. In particular, the present invention includes methods and processes for the quantification of light transmission through films of liquid crystals based on quantification of transmitted or reflected light.

The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not required to practice the present invention. Nevertheless, it is contemplated that ordered nanostructured substrates impart order to thin films of liquid crystal placed onto their surface. These ordered films of liquid crystal preserve the plane of polarized light passed through them. If the liquid crystal possesses a well-defined distortion – such as a 90 degree twist distortion—then the liquid crystal will change the polarization of the transmitted light in a well-defined and predictable manner. It is further contemplated that ordered films (e.g., areas of homeotropic orientation) of liquid crystal differentially absorb (relative to randomly ordered films of liquid crystal) specific wavelengths of light.

Accordingly, the present invention contemplates the use of plate readers to detect light transmission through an LC assay device when viewed through cross polars, the transmission of light through an LC assay device illuminated with a suitable wavelength of light, or reflection of light (*i.e.*, polarized light or non-polarized light of specific

and LC assay device are configured so that light provided from the plate reading device which is passed through or reflected from at least one surface of the LC assay device is detected by a detection unit of the plate reading device. Suitable detecting units include CCDs and photomultiplier tubes.

Commercially available plate readers that may be modified according to the present invention include, but are not limited, to those available from Nalge Nunc International Corporation (Rochester, NY), Greiner America, Inc. (Lake Mary, FL), Akers Laboratories Inc., (Thorofare, NJ), Alpha Diagnostic International, Inc. (San Antonio, TX), and Qiagen Inc. (Valencia, CA).

VI. Non-specific Detection Following Specific Capture

In some embodiments, the assays of the present find use for the non-specific detection of an analyte following specific capture. In these embodiments, the analyte is captured by a capture substrate (e.g., a PDMS stamp or bead) displaying a recognition moiety that interacts with the analyte. The analyte is then transferred to a detection substrate to which the analyte non-specifically binds. The presence of the analyte on the second substrate is detected by contacting the second substrate with a liquid crystal. Areas of disorder or order within the liquid crystal are indicative of the presence of analyte. As above, a variety of methods are useful for determining whether there is a changes in the orientation of the mesogens of the device. In some embodiments, the assay devices are configured with electrodes as described above so that the analyte can be transferred to a surface of the assay device by use of an electric current (e.g., by dielectrophoresis). The electrodes are also used to measured changes in electrical properties of the device (e.g., dielectric capacitance) as a result of changes in liquid crystal orientation.

In some preferred embodiments, the assays of the present invention are used for the detection of multiple species or genera of animals to a pathogenic organism. As a non-limiting example, antibodies specific West Nile Virus have been detected in samples collected from horses, mallard ducks, pigeons, rabbits, and mice. It will be recognized that these assays find use for testing samples from avian species such as crow, blue jay, eagles, sparrows and the more than 150 species of birds present in the US that are known to be infected with West Nile Viral, horses, humans, small mammals such as dogs and cats and other companion animals, rodents such as mice and rats, etc., and other wildlife such as raccoons, skunks, felines, canids, etc.

stamp substrate surface to the detection substrate. In preferred embodiments, the compound used to functionalize the surface of the detection substrate displays a stronger affinity for the analyte than does the recognition moiety so that the analyte is detached from the recognition moiety and transferred to the detection substrate.

In still further embodiments, the analyte is captured on a bead that displays a recognition moiety. As described above, the beads may be formed from latex, polymers, agarose, or other materials and in some preferred embodiments are magnetic. In some embodiments, the analyte is then transferred to the detection substrate. The transfer may be accomplished in a variety of ways. In some embodiments, the analyte is eluted from the beads either directly onto the detection substrate or eluted and the transferred to the detection substrate by a method such as spotting. In other embodiments, the beads exposed to analyte are contacted with the detection substrate so that the analyte is transferred to the detection substrate. As described above, in some embodiments, the detection substrate surface is functionalized with a moiety with a stronger affinity for the analyte than the recognition moiety on the bead so that the analyte is transferred to the detection substrate. In some embodiments, the signal from the analyte is amplified by binding one or more additional molecules to the analyte prior to elution. For example, if the analyte used is an antibody, a secondary anti-species antibody (e.g., and anti-Fc antibody for a particular species or rabbit-anti-human antibody, mouse-anti-human antibody, mouse-anti-rabbit antibody, etc.). Enzyme-antibody conjugates, analyte specific second antibodies, gold sol particles and other molecules and molecule systems may also be utilized. Where nucleic acids are being detected, the analyte detection assays outlined herein may follow an amplification method such as PCR.

A variety of detection substrates find use in the assays of the present invention, including the functionalized substrates described in detail above. In some preferred embodiments, the detection substrate comprises a rubbed polyimide or a polyimide that homeotropically orients a liquid crystal.

In some embodiments, after transfer of the analyte to the detection substrate, a liquid crystal is applied to the detection substrate so that the presence of the binding partner on the detection substrate can be detected. A variety of liquid crystal-forming substances can be used, including those listed above. In some preferred embodiments, 5CB is used. In some embodiments, the detection substrate is used to form an optical cell with another substrate and the liquid crystal is applied to a chamber formed by the two substrates.

recognition moiety-lipid complex can then be detected by transferring the complex to a substrate if necessary and then contacting the substrate with a liquid crystal. The present invention is not limited to any particular mechanism of action. Indeed, an understanding of the mechanism of action is not necessary to practice the present invention. Nevertheless, it is contemplated that the lipid portion of the complex provides homeotropic orientation to the portion of the liquid crystal in contact with the lipid. The homeotropic orientation can be detected by the methods described above. It will be recognized that it is not necessary that the substrate itself orient the liquid crystal. Thus, these assays can utilize low-cost simple substrates that do not provide an anisotropic surface or surface that is otherwise derivatized with an organic layer. Of course, the substrates have anisotropic surfaces or derivatized surfaces as described above if desired.

In other embodiments, the lipid-recognition moiety complexes are used as secondary binding agents to detect an analyte-recognition moiety complex. For example, an analyte may first be contacted with a first recognition moiety. In some embodiments, the first recognition moiety is a ligand for a second recognition moiety complexed with a lipid. The analyte-first recognition moiety complex is then contacted with the second recognition moiety-lipid complex so that the second recognition moiety binds to the first recognition moiety, thus labeling the analyte-first recognition moiety complex with the lipid. The presence of the lipid can then be detected as described above. In some preferred embodiments, the first recognition moiety is fused to either avidin or biotin so that a lipid complex comprising either avidin or biotin can be used as the secondary binding agent. In other embodiments, if the first recognition moiety is an antibody, the second recognition moiety can be protein A or an antibody that binds to the first antibody, for example, to the Fc region.

VIII. Kits

In some embodiments, the present invention provides kits for the detection of analytes. In preferred embodiments, the kits comprise one or more substrates as described in detail above. In some embodiments, the kits comprise capture and detection substrates. In some preferred embodiments, the capture substrates are beads or stamps. In further embodiments, the kits comprise a substrate that can be used in conjunction with the detection substrate to assemble a liquid crystal cell. In some embodiments, the kits comprise a vial containing mesogens. In still other embodiments, the kits comprise at least one vial containing a control analyte or analytes. In still other embodiments, the kit

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); C (degrees Centigrade); U (units), mU (milliunits); min. (minutes); sec. (seconds); % (percent); kb (kilobase); bp (base pair); PCR (polymerase chain reaction); BSA (bovine serum albumin).

Example 1

Immobilization of Antibodies on Substrates

This example describes different methods for immobilizing antibodies on substrate. Five different immobilization strategies were evaluated:

1) HEXA: adsorption of Protein A, then the West Nile Virus monoclonal antibodies (WNV Mabs) onto a hydrophobic monolayer formed from $CH_3(CH_2)_{15}SH$ (HEXA)on the surface of a gold film. The surface was blocked with BSA after immobilization of the antibody.

2) SPDP: covalent attachment of WNV Mabs to a monolayer formed from 2-mercaptoethylamine (2-MEA) on a gold film by using the sulfhydryl-reactive (protein) and amine-reactive (monolayer) heterobifunctional cross-linker N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). The surface was blocked with BSA after immobilization of the antibody.

3) PMPI: covalent attachment of Ras polyclonal antibodies (Ras Pabs) to a monolayer formed from 11-mercaptoundecanol (11-MU) on a gold film by using a sulfhydryl-reactive (protein) and hydroxyl-reactive (monolayer) heterobifunctional cross-linker N-(p-maleimidophenyl)isocyanate (PMPI). The surface was blocked with BSA after immobilization of the antibody.

4) DSS: covalent attachment of Ras Pabs to a monolayer formed from 2-MEA on a gold film by using an amino-reactive homobifunctional cross-linker disuccinimidyl suberate (DSS). The surface was blocked with BSA after immobilization of the antibody.

5) Adsp. : direct adsorption of WNV Mabs onto a thin film of polyurethane spin coated on the surface of gold. The surface was blocked with BSA after immobilization of the antibody.

homeotropic response to WNV is unique to the presence of specifically captured virus on the surface.

Next, it was necessary to confirm that the observed response in the topography-free region was a response to specifically captured WNV on the surface. Antibodies to SLE, Dengue, LACV and WNV were deposited on the same planar substrate. WNV stock was rocked across the four regions for 17 hours at 35°C. The cell was then observed through crossed polar lenses. The Dengue and LaCV antibody regions displayed brightly colored and disordered LC, indicating no binding of the WNV to non-specific antibodies. The WNV antibody region displayed a complete homeotropic circle, indicating bound virus. In the SLE antibody region, there was a small area of homeotropic alignment, signifying a slight cross-reaction of the WNV to the SLE antibodies.

Several observations were made. First, the homeotropic response of the liquid crystal to WNV is striking and unambiguous. The entire region of the surface that was exposed to the droplet containing WNV assumed a homeotropic orientation. Second, in contrast to the region of the surface presenting antibodies to WNV, the regions presenting antibodies to SLE, Dengue and LACV did not cause a homeotropic orientation of the liquid crystal. It is noted that a small area of the surface presenting the antibodies to SLE did cause homeotropic alignment, however, it is very small as compared to the area presenting antibodies to WNV, and it was clearly distinguishable from the area presenting antibodies to WNV. In summary, these results clearly demonstrate that the homeotropic response of the liquid crystal to WNV is a response to virus that is specifically captured on the regions of the surface presenting antibodies to WNV.

These results were obtained using an optical cell that comprised a second surface of glass treated with OTS. Because the liquid crystal orients homeotropically on the OTS-treated glass surface, the possibility that the homeotropic response of the liquid crystal to bound virus was caused by the OTS-treated glass slide in combination with a lack of orienting influence of a virus-decorated surface was considered. To address this possibility, an optical cell was prepared from two polyurethane surfaces decorated with virus. Homeotropic anchoring of the liquid crystal was observed when virus was captured by the antibodies on the polyurethane surface. From this result it can be concluded that the homeotropic orientation of the liquid crystal on the virus-decorated surfaces is a response to WNV and not caused by the OTS-coated glass slide. Additionally, a planar gold substrate was functionalized with $C_{16}SH$, then WNV monoclonal antibodies and then treated with WNV. The planar gold substrate was then paired with an OTS slide to create an optical

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Date: 10 févr. 2005

Recipient: IB

Address: Bureau international de l'OMPI 34, chemin des Colombettes Geneve 20 1211 CH

procedure. Instead of incubating the WNV as a stationary 20 µl drop on the substrate, a PDMS "pool" was used to contain a larger volume (300 µl) of WNV on top of the substrate. The WNV was then rocked continuously using a bi-directional rotator. This change in procedure led to a reduction of binding time from 3 hours to 2 hours. To determine the effect of reducing the spot size of the antibody, the antibody volume (to be immobilized) was decreased from 20µl to 5µl. By combining these three methods, increasing temperature, rocking, and reducing the surface area of immobilized antibody, the binding time was decreased from 2 hours to 1.5 hours. It should be emphasized that this is the only incubation time required for the assay. Once the virus and antibody have interacted, the liquid crystals are added and they assume their preferred orientation within seconds. Read out is immediate. The orientation is stable and the assay need not be read immediately. This does not represent a fixed time requirement. These end points were set as 100 % homeotropic alignment. Partial alignment has been noted before the 1.5 hr point. These results provide unambiguous evidence that liquid crystals can be used to detect WNV bound to a substrate coated with antibodies to WNV via the homeotropic response of the liquid crystal.

Example 5

Demonstration of Detection of St. Louis Encephalitis Virus (SLE)

To assess the generality of the method of reporting viruses other than WNV via the homeotropic response of the liquid crystal, an experiment was performed to determine if SLE could be detected via the response of liquid crystal to SLE captured on a surface presenting antibodies to SLE. Assays for SLE were performed essentially as described above for WNV. In each case when SLE virus was tested in the assay, areas of homeotropic alignment were observed on the edge of the slide, outside of the diagnostic zone but in the direction of the PBS wash. It appears as though the antigen-antibody complexes that formed on the diagnostic surface were washed away during the PBS rinse and only some residual remained near the edge of the slide to be visualized by the homeotropic alignment of the liquid crystals. This did not happen with the WNV assay. These areas indicate that SLE can be detected via the homeotropic response of the liquid crystal. This result is important, because it suggests that the homeotropic response of liquid crystal is not restricted to WNV but can be exploited for detection of viruses other than WNV. It would

Additional control experiments were performed using whole blood from birds and horses. Chicken blood (with either heparin or citrate as an anti-coagulant) and horse blood was applied directly to the substrate which was functionalized with WNV antibodies. Incubation was overnight at 35° C. The results clearly demonstrated that there is no cross-reaction or non-specific binding with normal avian or equine whole blood samples. Additional results were obtained with negative sera from chicken/horse/mallard/ or with culture media. Additionally, six mosquito homogenates from the NY State Dept of Health were that have previously shown to be negative for WNV by Taqman PCR were obtained and tested. These homogenates also tested negative with the LC assay and did not show any evidence of non-specific binding to the surface. This experiment demonstrates the ability to use concentrated biological samples with little processing in the assays of the present invention.

Example 8

Homeotropic Orientation by Cells

This example demonstrates homeotropic orientation by tissue culture cells. Tissue culture cells are allowed to attach to the surface of a glass slide. The surface is washed and mesogens are placed on the surface. Areas of the surface occupied by cells appear uniformly dark. Areas not occupied by cells regions display disordered and brightly colored LC.

Example 9

Detection by Stamp Transfer

This example describes the detection of antibodies transferred from a stamp substrate comprising a ligand to a detection substrate. To form the stamp substrate, 10 parts elastomer to 1 part curing agent from the Sylgard 184 Elastomer Kit (Dow Corning) is mixed together, degassed in a vacuum desiccator, and cured into PDMS at ~65^{0}C for 1 hour. The PDMS stamps are cut out from the PDMS cured to a Fisher's Finest Glass Microscope Slide. The PDMS stamps are then rinsed with ethanol and dried with nitrogen to clean them. The PDMS stamps are then plasma ashed in an oxygen plasma (200mTorr O_2 backfill pressure) at 275 Watts for 4 minutes to oxidize the surface of the PDMS producing a surface similar to glass. The PDMS stamps are then submerged in a 2%APES/98% dry

The results are presented in Figure 2. In this procedure, protein E is covalently bound to the DSS chemistry on the PDMS stamp. The protein E in turn captures WNV antibodies if present in the serum incubation droplet. Even if there are no WNV antibodies in the serum (in this case the negative serums or controls on the right of Fig. 2) there is assumed to be some non-specific absorption to the PDMS stamp surface, thus the need for using the Triton rinse to remove non-specific absorbed entities. When the PDMS stamp is brought into contact with the ATP treated gold surface the protein E stays covalently bound to the DSS chemistry on the stamp and the captured WNV antibodies, if present, transfer to the ATP chemistry due to the physics of a stronger bond. Referring to the pictures in Fig. 2, the two optical cells on the left, as viewed through crossed-polarizers, were stamped with PDMS that had sera positive for the WNV antibodies incubated on them. The two pictures on the right of Fig. 2 show the control optical cells, as viewed through crossed-polarizers, that were stamped with PDMS that had sera negative for WNV antibodies incubated on them. When WNV antibodies are present, the stamps that captured WNV antibodies on them transfer to the ATP treated gold causing a circular (from the shape of the incubation droplet) disruption pattern in the liquid crystal optical cell, see pictures on the left. The control stamps that have not captured antibody demonstrate homeotropically aligned liquid crystal in the optical cell (see pictures on the right of Fig. 2).

Example 10

Affinity Contact Printing (αCP) to Report a Specific Antibody from a Population

This experiment demonstrates the capture of a specific antibody from a mixture of two antibodies in solution. A six button PDMS stamp, see Figure 4, was plasma ashed in an O_2 cloud for 8 minutes to oxidize the surface of the PDMS. The stamp was then placed in a 2%APES in dry acetone solution for 2 minutes while stirring. The stamp was then placed in acetone for 5 minutes while stirring. The stamp was then rinsed with acetone, dried with nitrogen, and placed in a 100°C oven for 30 minutes. The stamp was then placed in a 1mM DSS solution for 1 hour while stirring. The stamp was removed from the DSS, rinsed with methanol, and dried with nitrogen. On three of the buttons on the six button PDMS α-stamp, 20μl drops of 0.25μM protein E were incubated overnight at 4°C. The remaining three buttons had 20μl drops of 0.25μM biotinalated BSA incubated on them overnight at 4°C. The proteins were rinsed off with water and the α-stamp was dried with nitrogen. Two of the three protein E functionalized buttons and two of the three biotinalated BSA

non-load wheel current: 0.065 amps

loaded wheel current: 0.055 amps

left height readout: 2.530

right height readout: 2.530

The following materials were deposited in sequence from droplet onto the wafer. A two hour incubation period was used for each addition:

Protein A: 1 mg/ml

Bovine serum albumin: 0.1 mg/ml

Mouse IgG: 1 microMolar

The surface thickness of the wafer, calculated from ellipsometric readings following each binding step, are listed below:

Polyimide coated wafer	21.0, 21.1
Protein A	23.4, 22.9, 22.9
BSA	22.3, 21.8, 22.2
Mouse Antibody	24.9, 24.2

These readings demonstrate the binding of Protein A to the polyimide surface and the subsequent binding of Mouse antibody to the Protein A.

Example 12

Detection of molecular interactions on polyimide surfaces by liquid crystals

Glass slides were scrubbed with 1-Methyl-2-Pyrrolidinone(NMP), spin cleaned with NMP at 1700 rpm and spin coated with 1.0% solids polyimide SE-7210 at 1700 rpm. The slides were pre-cured at 85 C for 10 min and subjected to a final cure at 180 C for 15 minutes. The following materials were added by droplet incubation in the order listed:

Protein A: 1 mg/ml

BSA 0.1 mg /ml

Mouse antibody 1 microMolar

Slides were washed with phosphate buffered saline in between incubations. They were dried with a stream of nitrogen and formed into a cell by the addition of a top slide. Liquid crystal ZLI-1221 was added to the space between the glass slides.

The binding of mouse antibody to the immobilized protein A present on the polyimide surface was reported by a change in orientation of the liquid crystals. Areas with only Protein A or with Protein A plus BSA appeared dark to the naked eye when visualized

samples with Protein A + BSA+ Mouse antibody. The area appeared white under cross polars. There was no significant binding of the rat antibody to the Protein A. this indicates specificity of binding on a polyimide surface.

Example 14

Polyimide surfaces can specifically detect target molecules using a "sandwich" technique and the sensitivity of the system can be adjusted by controlling the amount of receptor present on the surface

Glass slides were coated with polyimide and rubbed using the standard protocol described above. The slides were functionalized with the following reagents, using a 30 minute incubation period for each reagent. A series of dilutions of Protein A in the blocking agent fish gelatin of 1:99, 10:90, 25:75, and 50:50 were made to control the sensitivity of the surface to the target molecule

Protein A 1.0 mg/ml

Fish Gelatin 0.1% dilution of a 30% stock solution.

Anti-biotin 100 micrograms/ml

Biotin (100micrograms/ml) was added to each functionalized area. The secondary anti-biotin antibody (100 micrograms /ml) was applied. Control regions were included that were processed as follows:

1. Protein A + fish gelatin
2. Protein A + fish gelatin + antibody 1
3. Protein A + fish gelatin +antibody 1 + biotin

Controls 1 and 2 appeared dark when viewed between cross polars, indicating that liquid crystals were uniformly aligned on the surface. Control 3 showed a minimum of disruption, but appeared mostly dark between cross polars, incdicating uniform alignment of the liquid crystals. The degree of disruption did appear to increase as the ratio of Protein A to fish gelatin increased, indicating an increase in sensitivity of the surface. The regions exposed to the complete sandwich, Protein A+ fish gelatin+ antibody 1 + biotin + antibody 2, appeared white when viewed between cross polars, indicating disruption of liquid crystal alignment and therefore the binding of the target molecule, biotin. All ratios of Protein A to fish gelatin yielded similar results in the complete sandwich indicating that concentrations lower than the 1:99 ration could be used to tune the assay.

Areas incubated with 1.0 microMolar or 0.1 microMolar mouse antibody showed significant disruption in the liquid crystal film. The area incubated with 0.01 microMolar mouse antibody did not cause disruption in the liquid crystal film. The rat antibody did not cause disruption. The number of antibodies bound to the surface area for the 0.01 microMolar assay may be below the threshold needed to cause disruption in the liquid crystal film. Sensitivity may be increased by decreasing the functionalized surface area.

Example 18

Use of Fish Gelatin to Minimize Protein A binding sites

Four polyimide coated and rubbed slides were blotted with the materials listed below. The slides were blotted using a 30 minute incubation time for each of the materials. A dilution series was created using 1:99, 10:90, 25:75 and 50:50 ratios of Protein A and the diluted Fish Gelatin. Each slide was blotted with one of the four mixed Protein A/Fish gelatin dilution samples. Subsequent additions of primary antibody (anti-biotin), biotin, and secondary antibody (anti-biotin) were added to the specified areas. The coating of slides and the rubbing procedures were identical to those used in the examples above. Reagents:

Protein A: 1.0 mg/ml

Fish Gelatin: 0.1% dilution of 30% stock

Anti-biotin: 100 micrograms/ml

Biotin antigen: 100 micrograms/ml

ZL1-1221

Slight disruption was seen when the primary antibody binds to Protein A for 50:50 and for 25:75 samples. For 10:90 ratios, the disruption is less than above and for 1:99, it is even less.

By diluting the Protein A with fish gelatin it is possible to limit the amount of primary antibody. This is an approach to limit the sensitivity of the system. This allows the rubbed polyimide to be used for systems in which the goal is to create an assay for the detection of an antigen as well as systems for the detection of antibody. Based on these results, it appears that a concentration even lower that 1:99 could be used and may result in even less disruption with the primary antibody and antigen, while still giving good disruption in response to the binding of the secondary antibody.

measuring differential capacitance using an *off-the-shelf* chip (e.g., MS3110 Universal Capacitive Readout from MicroSensors Inc, Costa Mesa CA).

The sensitivity of the detection system depends on the resolution of the device used for measurement of the capacitance. Both commercially available LCR meters and *off-the-shelf* differential capacitance measurement chips have a resolution on the order of 0.1 fF in10 pF level. An order of magnitude estimate of 0.1fF resolution for interdigitated electrodes described above corresponds to a total of 10 virons on a 500 μm x 500 μm area. This result predicts that by using an electrical detection system it is possible to detect 10 viron particles bound to the surface.

Arrays of interdigitated co-planar electrodes are designed based on the calculations described above. In preferred embodiments, electrodes are in the micrometer range and fabrication of these electrodes will be performed by using standard photolithographic methods of patterning followed by a lift off process. These electrode arrays are fabricated on commercially available glass substrates.

Estimation of the change in capacitance between planar and homeotropic orientation of liquid crystals

Using electrodes fabricated as described above, experimental measurements are preformed to measure the capacitance of the liquid crystal anchored on the electrodes in known orientations. These measurements are performed by coating the electrodes with LC alignment films that give rise to known orientations of LCs. A thin (20 nm-thick) LC alignment layer (Nissan SE 7210) is coated onto two glass substrates (one with optimized electrodes and the other without electrodes) and buffed to create anisotropy in the surface morphology that aligns the LC material in a predetermined azimuthal direction perpendicular to the electrode fingers. An approximately 25 μm thick optical cell is fabricated by clamping these two substrates, separated by a Mylar film at each end, together. The liquid crystal 4-cyano-4'-pentylbiphenyl (5CB) is injected in to the gap between the substrates in isotropic phase (40°C) and cooled down to room temperature. The capacitance is measured between the electrodes using a precision LCR meter (HP 42841, Agilent Technologies).

A similar cell is prepared using the homeotropic alignment layer (Nissan SE 7511L) and the capacitance between the electrodes is measured. A comparison between these two

Example 20

Use of capacitance measurements in combination with liquid crystals to achieve electrical detection of virus

The measurement of capacitance

Using surfaces and electrode geometries validated as described in Example 19, a high precision LCR meter is used to measure the change in capacitance between electrodes supporting a film of liquid crystal on a surface without bound virus and a film of liquid crystal upon a surface with bound virus. In order to determine the change in capacitance induced by the orientational transition, five optical cells are constructed:

1) untreated polyurethane(PU) surface;
2) PU surface treated with antibody to VSV-I;
3) PU surface treated with antibody to VSV-I and a non-specific virus such as herpes simplex;
4) PU surface treated with antibody to VSV-I, and VSV-I virus; and
5) PU surface treated with a non-specific antibody (e.g., anti-biotin IgG) and VSV-I virus.

A comparison of capacitance between two electrodes in all five types of cells provides a quantitative measure of the change in capacitance upon specific binding of VSV-1 to the surface. When implemented in the final device used for assay measurements, we will employ a differential capacitive measurement system similar to MS3110 Universal Capacitive Readout (MicroSensors Inc. CA) for measurements of capacitance. A system like the MS3110 permits facile measurement of the change in the differential capacitance between the electrodes treated with antibody targeted to VSV and the control surfaces (e.g. the surfaces presenting non-specific antibody). The output voltage of such a system is a linear function of the change in the differential capacitance between two inputs fed to it. A series of virus concentrations are tested to establish the relationship between the concentration of bound virus and the change in capacitance of the liquid crystal film.

Selection of optimal liquid crystalline materials

of high or low electric field strength. If the particle is more polarizable than the suspending medium, it moves toward the region of strong electric field and if the particle is less polarizable than the medium, it moves toward the region of low electric field. The magnitude of dielectrophoretic force depends also on, besides the dielectric properties of the particles and the medium, the gradient of the electric field and the size of the particle. For a particle of radius r_p in an electric field with

gradient $\nabla|E_{rms}|^2$, the average dielectrophoretic force is given by,

$$F_{DEP} = 2\pi r_p^3 \varepsilon_m Re[K_e] \nabla|E_{rms}|^2$$

where ε_m is the permittivity of the medium, E_{rms} is the root mean square electric field intensity and $Re[K_E]$ is the real part of Claussius-Mossoti factor given as,

$$K_E = \frac{(\varepsilon^*_p - \varepsilon^*_m)}{(\varepsilon^*_p + 2\varepsilon^*_m)}$$

where ε^*_p and ε^*_m are effective dielectric permittivity of the particle and the medium, respectively. The direction of the dielectrophoretic force is determined by the relative sign of $Re[K_E]$ which depends on the relative conductivity and permittivity of the particle and the medium. For example, for a spherical virus particle suspended in a physiological medium such as TSE with $\sigma_m = 600\ mSm^{-1}$, *and* $\varepsilon_m = 80\varepsilon_0$, the single shell model yields $Re[K_E] = -0.46$ at 10 MHz. This result indicates that the virus particle will move toward the region of lower electric field at 10 MHz. This is termed "negative dielectrophoresis". The dieletrophoretic force exerted on a virus particle of radius 250 nm, in a field gradient defined by hyperbolic electrodes separated by 10 μm with AC field of strength 5 V_{pp} between them, located at the edge of the electrode is approximately 3 pN. For comparison, the force associated with Brownian motion of the particle in a medium is of the order of $F_B = K_B T/(2 r_p)$, where K_B is the Boltzmann constant and T is the absolute temperature. Thus at room temperature, the force experienced by the virus particle is on the order of 10^{-2} pN. These results clearly indicate that the dieletrophoretic force is *at least two orders of magnitude stronger* than the thermal force exerted on the particle, which is responsible for the diffusive transport of the virus particles.

as Minimum Essential media with 10% fetal calf sera as well as various animal and human sera and standard viral transport media will be examined for their dielectric properties and their effects on the dielectrophoretic force exerted on the virus. Combinations of variables such as strength and frequency of the applied AC field, and ionic strength of the suspending medium, that yield the maximum response in the least amount of time and with the least amount of sample will be identified. To monitor these events in real time the virus particles are labeled. A method recently published by Akin et al., Real-time virus trapping and fluorescent imaging in microfluidic devices, Nano Letters 4: 257-259 (2004) is utilized which used a lipophilic carbocyanin dyes to label the envelope of vaccinia virus. The dyes (DiOC63 and DIL, Molecular Probes, CA) label the lipid membrane and capsid proteins of the virus and permitted visualization of surface bound vaccinia by digital epifluorescence microscopy at 400X magnification.

Antibody functionalized surfaces with integrated microelectrodes (Figure 3) fabricated as described above are exposed to a 20μL droplet of labeled VSV-I. An AC electric field is applied across the electrodes to induce dielectrophoretic motion of the virus particles to the surface in the region between the electrodes. VSV-1 binds to the antibodies on the surface. The field is turned off and the unbound virus is washed from the surface. These events are monitored in real time using a Zeiss-Axiovert 200 M microscope.

The optimal parameters for the dielectrophoresis of VSV-1 are identified by observation of the intensity of fluorescence seen within the center region of the electric field. The intensity of the fluorescence is correlated with the known concentrations of virus in the applied sample. The strength and frequency of the applied field that permit detection of the least amount of virus in under 5 minutes are chosen for testing against various suspension media of physiologic ionic strength. Such media include typical clinical materials such as serum, nasal swab fluid, and viral transport media to determine how the strength and frequency required for viral transport will be affected by these common sample fluids.

Example 22

Use of Beads for Capture of an Analyte Followed by Non-specific Detection

Preparation of beads. Sera-Mag beads (0.8μM in diameter) were functionalized with either 0.4mg/mL EDC (Aldrich) or 1.1 mg/mL Sulfo-NHS (Pierce). First, 27 μl of 5% Sera-Mag beads were diluted in 1mL of the functionalizing agent. Reactions were carried

Figure 8. A digitized image taken with two Polaroid filters at cross polar configuration at 0 °. Polyimide 7511L slides were prepared from 40% undiluted stock. These slides, in absence of protein, assume homeotropic alignment as shown in dark background. Upon protein binding, homeotropic alignment is disrupted and shown in white background. In the top row, from left to right, are elution samples from aF1pAb beads initially blocked with RNase A treated with BSA 400ng/mL, F1 500pg/mL and 2.5ng/mL. The bottom row is 25, 100, and 400ng/mL concentrations of F1. After initial protein binding in binding solution PBS/T/H/N, aF1pAb beads were washed in PBS/Tween 0.05%, 200mM additional NaCl (PBS/T/N) wash solution.

An additional example demonstrates how this approach one can determine presence and quantity of the target molecule. Figure 9 below demonstrates that with increasing F1 concentration, the disruption of homeotropic alignment increases as indicated by increased white signal against aligned LC black background on homeotropic aligning polyimide surfaces. This type of assay can be used to quantify levels of the F1 antigen.

In Figure 9, the digitized figures were taken by polarized microscope with cross polar filt (0 °). Polyimide 7511L slides were prepared from 40% undiluted stock. In absence of prote these slides assume homeotropic alignment as shown in by the dark background. Upon prot binding homeotropic alignment is disrupted (indicated by a white background). The followi samples were analyzed: elution buffer, BSA (200ng/mL), and F1 elutions of beads exposed to 10, 50 and 200 ng/mL concentrations of F1. The conditions were as described above. Th experiments were carried in triplicate and Figure 9 had intermediate levels of signal with respect two other replicates (not shown). As can be seen, it was possible to detect 5 ng/mL analyte.

Figure 10 is a graphic representation of luminosity index for the experiment depicted Figure 9. These experiments were carried in triplicate and figure chosen above have intermedi levels of signal with respect to two other replicates. Elution buffer was also applied to one area a used as reference for Luminosity index shown in the graph below.

In further experiments, a rubbed polyimide surface was utilized. Figure 11 shows digitized image taken with a polarized microscope with cross polar filters (0 degrees). Polyimi SE 7210 1.5% slides were rubbed at 2.55ml/m setting at 4.0cm/s table speed with a wheel speed 343rpm. Figure 11 shows the results of elutions to the polyimide surface from functionali aF1pAb beads that were treated with various concentrations of F1 or BSA as control. Af washing, the beads were treated by the addition of rabbit anti-mouse IgG (5µg/mL) and anti-mo FC IgG (6µg/mL). The complex was eluted off of the beads with 10µl acid elution of 0.1M glyc pH 2.3. Elution samples were neutralized by addition of 1µl 1 M Tris pH 7.5. The samples w contacted with the polyimide substrate and an optical cell was constructed by placing a my

Chicken Serum). Since the functionalized beads were blocked with BSA, it is most likely the BSA blocker that creates the signal seen in the first spot.

Example 23

Homeotropic orientation of liquid crystals by cells

Tables 2 and 3 present the results of experiments in which different liquid crystals were surveyed for their ability to be homeotropically oriented by cultured cells. Many liquid crystals align homeotropically in response to phospholipids and cholesterol. Phospholipids (2 ul; 0.01 M in chloroform) were applied to discrete marked areas on glass slides. The phospholipids had dioleoyl alkyl chains and the following headgroups: phosphatidylserine (DOPS), phosphatidylglycerol (DOPG), phosphatidylethanolamine (DOPE), phosphatidylserine (DOPS), phosphatidic acid (DOPA), and lysophosphatidylcholine (DOLPC). After the solvent dried, optical cells were assembled with liquid crystals applied nematically and heated to isotropy. Homeotropic alignment was confirmed by conoscopic analysis. Chol = cholesterol; C = cholesteric alignment; Bkg= background alignment; U= unaligned; H= homeotropically aligned; ND indicates not done due to background. 4OCB, 4 -octyl-4-biphenyl-carbonitrile (Aldrich); 6CHBT, 1-(*trans*-4-hexylcyclohexyl)-4-isothiocyanato-benzene. All other liquid crystals are from EM Industries/Merck.

Table 3: Investigation of phospholipid influence on liquid crystal alignment.

Liquid Crystal	Bkg	DOPS	DOPG	DOPC	DOPE	DOPA	DOLPC	Chol
4OCB	H	ND	ND	ND	ND	ND	ND	ND
5CB		U	H	H	H	Planar	H	U
6CHBT		U	H	H	H	H	H	U
E7		Twisted planar	H	H	H	Twisted planar	H	H
ZLI-1221		H	H	H	H	H	H	H
ZLI-1557		H	H	H	H	H	H	H
ZLI-2222		H	H	H	H	H	H	H
ZLI-3225		U	H	H	H	H	H	H
ZLI-3497	H	ND	ND	ND	ND	ND	ND	ND
ZLI-4431	Chol	U	U	U	U	U	U	U
ZLI-4446	H	ND	ND	ND	ND	ND	ND	ND
ZLI-5070		Twisted planar	H	H	H	H	H	H
MLC-6080		U	H	H	H	H	H	H
MLC-6466		U	H	H	H	H	H	H
MLC-6710-080		U	H	H	H	H	H	H
MLC-15700-000		H	H	H	U	H	H	U
TL205		U	H	H	H	H	H	H

Example 24

Detection of analyte with tagged lipids

This experiment describes the detection of binding of liposomes labeled with biotin to the immobilized anti-biotin antibody using liquid crystals. Anti-biotin immobilized glass substrates were prepared as follows. PrecisionCT slides (Bioslide Technologies; Cat# BSP-SC02-C) cleaned in plasma asher (20min, 275 watts, 200 millitor) were immersed in 2% APES (3-Aminopropyltriethoxysilane; Pierce) in dry acetone for 2min. Slides were transferred to pure acetone and stirred for 5min and rinsed with acetone to remove excess of silane. Slides were dried with nitrogen and kept at 110 C oven for 45 min. Slides were removed from oven and after they reached room temperature areas were marked on one side of slide. 1mg/ml BS3 (Bis (Sulfosuccinimidyl) suberate; Pierce) cross-linker was applied as 10ul drops on marked areas and incubated for 15min at room temperature. After rinsing excess cross-linker with water surfaces were dried with nitrogen gas. 100ug/ml and

sonicated in a bath type sonicator (Laboratory Supply company, Hicksville NY) at 55C to give a water-in-ether emulsion. The ether was then evaporated from the emulsion using a rotary evaporator, bath temperature 55C. After ether removal, the mixture formed a viscous gel, which on agitation, broke down to give a liposome suspension. After liposome formation, the two 50 micromol batches for sample and control liposomes respectively were combined for the next step.

The size of the liposomes was then reduced to a smaller and more uniform diameter by a process known as extrusion, wherein they were passed through a series of polycarbonate membranes of defined pore size. The extrusion chamber was heated to 60C, and the liposomes were passed five times through a 0.4 micron pore size polycarbonate membrane, and then five times through a 0.1 micron pore size polycarbonate membrane. The process of extrusion noticeably reduces the turbidity of the suspension.

The liposomes were then dialysed overnight at 4C against 1 liter of the suspension buffer so as to eliminate any residual traces of isopropylether. The liposomes were analyzed for their phospholipid content using the phosphorus assay of Bartlett, and were found to contain:

Sample (biotin) liposomes: 17.84 micromol phospholipid/ml

Control liposomes: 18.7 micromol phospholipid/ml

The liposomes were analyzed for their size using a Nicomp 380 particle sizer. The volume-weighted gaussian mean diameters were:

Sample (biotin) liposomes: 134 nanometer

Control liposomes: 157 nanometer.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in organic chemistry, materials science, chemical engineering, virology,

Claims

What is claimed is:

1. A method for detecting viruses comprising:

a) providing:

i) a sample suspected of containing of a virus;

ii) a detection device comprising a substrate comprising at least one detection region having a first virus recognition moiety immobilized thereon;

iii) mesogens;

b) contacting said detection region with said sample;

c) contacting said substrate with said mesogens, wherein the presence of said virus is indicated by a change in said mesogens over said detection regions and wherein said change is independent of the presence of an additional homeotropic director on said detection region.

2. The method of claim 1, wherein said change in said mesogens is selected from the group consisting of a change in color, a change in texture, a change in tilt, and homeotropic orientation.

3. The method of Claim 1, wherein said change in mesogens is detected by a method selected from the group consisting of visual detection, optical detection, spectroscopy, light transmission, and electrical detection.

4. The method of Claim 1, wherein said virus is selected from the group consisting of the following families: Adenoviridae, Arenaviridae, Astroviridae, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Iridoviridae, Filoviridae, Orthomyxoviridae, Papovaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Poxviridae, Reoviridae, Retroviridae, Rhabdoviridae, Togaviridae, Badnavirus, Bromoviridae, Comoviridae, Geminiviridae, Partitiviridae, Potyviridae, Sequiviridae, and Tombusviridae.

15. The method of Claim 1, wherein said plurality of detection regions have different virus recognition moieties bound thereto.

16. The method of Claim 1, wherein said detection device further comprises a second substrate arranged opposite said first substrate to form a cell.

17. The method of Claim 1, wherein said change in said mesogens is detected by viewing said detection device between cross polar lenses.

18. The method of Claim 1, wherein said detection region does not homeotropically orient mesogens in the absence of virus.

19. The method of Claim 1, wherein said sample is selected from the group consisting of biological fluids, tissue homogenates, feces, vesicular fluids, swabs of orifices or tissues, and media in which virus has been cultured or prepared.

20. The method of Claim 1, wherein said biological fluid is selected from the group consisting of cerebral-spinal fluid, urine, serum, plasma, nasal secretions, sputum, semen and saliva.

21. The method of Claim 1, wherein said homeotropic ordering is observed within 48 hours of the application of said sample to said detection region.

22. A device for the detection of a virus comprising a first substrate comprising at least one detection region having a first virus recognition moiety specific for said virus immobilized thereon, wherein said detection region does not homeotropically orient an added mesogen in the absence of said virus.

23. The device of Claim 22, wherein said first substrate comprises a plurality of detection regions.

32. A method for detecting a lipid membrane containing entity comprising:

a) providing:

i) a sample suspected of containing of an entity with a lipid membrane;

ii) a detection device comprising a substrate comprising at least one detection region;

iii) mesogens;

b) contacting said detection region with said sample;

c) contacting said substrate with said mesogens, wherein the presence of said biological entity with a lipid membrane is indicated by a change in said mesogens over said detection regions.

33. The method of claim 32, wherein said change in said mesogens is selected from the group consisting of a change in color, a change in texture, a change in tilt, and homeotropic orientation.

34. The method of Claim 32, wherein said change in mesogens is detected by a method selected from the group consisting of visual detection, optical detection, spectroscopy, light transmission, and electrical detection.

35. The method of Claim 32, wherein said entity is selected from the group consisting of a cell, a bacterium, a Mycoplasma, a virus, and a liposome or combinations thereof.

36. The method of Claim 32, wherein said substrate is selected from the group consisting of metal films, glass, silicon, diamond and polymeric materials.

37. The method of Claim 36, wherein said polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate.

38. The method of Claim 32, wherein said mesogen is selected from the group consisting of 4-cyano-4'-pentylbiphenyl, N-(4methoxybenzylidene)-4-butlyaniline and combinations thereof.

50. The method of Claim 32, wherein said biological fluid is selected from the group consisting of cerebral-spinal fluid, urine, serum, plasma, nasal secretions, sputum, semen and saliva.

51. The method of Claim 33, wherein said homeotropic ordering is observed within 48 hours of the application of said sample to said detection region.

52. The method of Claim 32, wherein said entity comprising a lipid membrane is a liposome displaying a ligand.

53. A device for the detection of an entity comprising a lipid membrane, said device comprising a first substrate comprising at least one detection region having at least one recognition moiety specific for said entity comprising a lipid membrane immobilized thereon, wherein said detection region does not homeotropically orient an added mesogen in the absence of said entity comprising a lipid membrane.

54. The device of Claim 53, wherein said first substrate comprises a plurality of detection regions.

55. The device of Claim 53, wherein said recognition moiety is selected from the group consisting of antigen binding protein and nucleic acid.

56. The device of Claim 54, where said plurality of detection regions comprise recognition moieties.

57. The device of Claim 56, wherein at least two of said plurality of detection regions comprise the same recognition moiety.

58. The device of Claim 56, wherein said plurality of detection regions are arranged in an array.

65. A method comprising:

a) providing a functionalized detection substrate treated to align mesogens, a stamp substrate displaying at least one recognition moiety, a biological test sample suspected of containing a binding partner for said recognition moiety, and mesogens;

b) contacting said test sample with said stamp substrate under conditions such that said binding partner can bind said recognition moiety;

c) contacting said detection substrate with said stamp substrate under conditions such that said binding partner to said recognition moiety is transferred to said detection substrate;

d) detecting the presence of said binding partner to said recognition moiety on said detection substrate by applying said mesogens to said substrate.

66. The method of Claim 65, wherein said biological sample is selected from the group consisting of whole blood, serum, cerebral spinal fluid, nasopharyngeal aspirate, and nasal secretions.

67. The method of Claim 65, wherein alignment of said mesogens by said detection substrate is disrupted by the presence of said binding partner to said recognition moiety.

68. The method of Claim 67, wherein said alignment is homeotropic.

69. The method of Claim 65, wherein said mesogens are not homeotropically aligned over areas of said detection substrate wherein said binding partner of said recognition moiety is present.

70. The method of Claim 65, wherein said detection substrate is used to form an optical cell.

71. The method of Claim 65, wherein said detecting is performed by analysis of said detection substrate with cross-polar lenses.

84. The method of Claim 65, wherein said stamp substrate comprises two or more recognition moieties in an array.

85. The method of Claim 65, wherein said ligand is bound by binding partners from a plurality of species or genera.

86. The method of Claim 65, wherein said mesogen is 5CB.

87. The method of Claim 65, wherein said stamp substrate comprises PDMS.

88. The method of Claim 65, wherein said detection substrate comprises obliquely deposited gold.

89. A kit comprising:

a) a stamp substrate displaying at least one recognition moiety;

b) a functionalized detection substrate that orients mesogens; and

c) instructions for using said substrates for detecting a binding partner of said recognition moiety.

90. The kit of Claim 89, further comprising a container of mesogens.

91. The kit of Claim 89, further comprising a container containing a secondary binding agent.

92. A system for detecting an analyte comprising:

a) a first substrate displaying a recognition moiety, wherein said recognition moiety interacts with said analyte;

b) a second substrate comprising a surface configured to receive said analyte interacting with said recognition moiety; and

c) a liquid crystal overlaying said second substrate.

104. The system of Claim 103, wherein said polyimide is selected from the group consisting of Nissan 7511L and SE 1211.

105. A method of detecting an analyte comprising:

a) providing a first substrate displaying a recognition moiety, a second substrate, mesogens, and a sample suspected of containing an analyte;

b) contacting said first substrate displaying a recognition moiety with said sample suspected of containing an analyte so that said analyte interacts with said recognition moiety;

c) transferring said analyte interacting with said recognition moiety to said second substrate; and

d) contacting said second substrate with said mesogens to detect the presence of said analyte on said second substrate.

106. The method of Claim 105, wherein said recognition moiety is selected from the group consisting of a protein, polypeptide, peptide, nucleic acid, carbohydrate, lipid, organic molecule and inorganic molecule.

107. The method of Claim 105, wherein said analyte is selected from the group consisting of a protein, polypeptide, peptide, nucleic acid, organic molecule, inorganic molecule, virus, liposome, bacteria, fungus, and cell.

108. The method of Claim 105, wherein said first substrate is selected from the group consisting of a stamp, a bead, and a column.

109. The method of Claim 105, wherein said second substrate is selected from the group consisting of silicon, glass, polymer, diamond, and metal.

110. The method of Claim 105, wherein said second substrate comprises a surface functionalized with a polyimide.

111. The method of Claim 110, wherein said polyimide is rubbed.

120. The method of Claim 105, wherein said transferring step further comprises the step of eluting said analyte from said first substrate.

121. The method of Claim 105, further comprising the step of contacting the analyte-recognition moiety complex with a secondary binding agent.

122. The method of Claim 121, wherein said secondary binding agent is selected from the group consisting of a peptide, polypeptide, protein, carbohydrate, and nucleic acid.

123. The method of Claim 121, wherein said secondary binding agent is avidin or biotin.

124. The method of Claim 121, wherein the presence of said secondary binding agent enhances the detection of said analyte after transfer to said second substrate.

125. The method of Claim 122, wherein said secondary binding agent is complexed with a lipid.

126. The method of Claim 125, wherein said secondary binding agent is displayed on a liposome.

127. A kit comprising:

a) a first substrate displaying a recognition moiety, wherein said recognition moiety interacts with an analyte;

b) a second substrate comprising a surface configured to receive said analyte interacting with said recognition moiety;

c) a vial containing mesogens; and

d) instructions for detecting said analyte.

128. The kit of Claim 127, wherein is said first substrate is selected from the group consisting of a stamp, a bead, and column media.

136. The method of Claim 135, wherein said polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate.

137. The method of Claim 129, wherein said mesogen is selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline) and combinations thereof.

138. The method of Claim 137, wherein said mesogen is 5CB.

139. The method of Claim 129, wherein said detection region comprises a recognition moiety.

140. The method of Claim 129, wherein said recognition moiety is selected from the group consisting of an peptide, polypeptide, protein, nucleic acid, carbohydrate, organic molecule, and inorganic molecule.

141. The method of Claim 140, wherein said protein is an antigen binding protein.

142. The method of Claim 129, wherein said substrate comprises a plurality of detection regions.

143. The method of Claim 142, wherein said plurality of detection regions display the same recognition moiety.

144. The method of Claim 142, wherein said plurality of detection regions display different recognition moieties.

145. The method of Claim 129, wherein said detection device further comprises a second substrate arranged opposite said first substrate to form a cell.

156. The device of Claim 155, wherein said polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate.

157. The device of Claim 151 further comprising mesogens, wherein said mesogens are selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline) and combinations thereof.

158. The device of Claim 151, wherein said detection region comprises a recognition moiety.

159. The device of Claim 158, wherein said recognition moiety is selected from the group consisting of an peptide, polypeptide, protein, nucleic acid, carbohydrate, organic molecule, and inorganic molecule.

160. The device of Claim 159, wherein said protein is an antigen binding protein.

161. The device of Claim 151, wherein said first substrate comprises a plurality of detection regions.

162. The device of Claim 161, wherein said plurality of detection regions display the same recognition moiety.

163. The device of Claim 161, wherein said plurality of detection regions display different recognition moieties.

164. The device of Claim 151, wherein said at least one electrode is selected from the group consisting of interdigitated, hyperbolic, triangular and rectangular electrodes.

165. The device of Claim 151, wherein said first substrate comprises at least two electrodes.

175. The method of Claim 174, wherein said analyte non-specifically interacts with said surface comprising polyimide.

176. The method of Claim 175, wherein said surface comprising polyimide displays a recognition moiety.

177. The method of Claim 174, wherein said recognition moiety is selected from the group consisting of a protein, polypeptide, peptide, nucleic acid, carbohydrate, lipid, organic molecule and inorganic molecule.

178. The method of Claim 174, wherein said mesogens are selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline).

179. The method of Claim 174, wherein said polyimide is rubbed.

180. The method of Claim 179, wherein said polyimide is selected from the group consisting of Nissan 7210, Nissan 3510, Nissan 410, Nissan 3140, Nissan 5291, and Japan Synthetic Rubber JALS 146-R19.

181. The method of Claim 174 wherein said polyimide homeotropically orients said mesogens.

182. The method of Claim 181, wherein said polyimide is selected from the group consisting of Nissan 7511L and SE 1211.

183. The method of Claim 174, wherein the presence of analyte is indicated by a disordered liquid crystal that appears white or bright when viewed through cross polar lenses and areas where analyte is not bound remain ordered and appear dark when viewed through cross polar lenses.

192. The method of Claim 189, wherein said analyte is selected from the group consisting of a protein, peptide, polypeptide, nucleic acid, organic molecule, inorganic molecule, virus, bacteria, liposome, cell, and fungus.

193. The method of Claim 190, wherein said substrate is selected from the group consisting of metal films, glass, silicon, diamond and polymeric materials.

194. The method of Claim 193, wherein said polymeric materials are selected from the group consisting of polyurethane, PDMS, polyimide, polystyrene, polycarbonate and polyisocyanoacrylate.

195. The method of Claim 186, wherein said mesogen is selected from the group consisting of E7, MLC, 5CB (4-n-pentyl-4'-cyanobiphenyl), 8CB (4-cyano-4'octylbiphenyl), BL093, TL 216, ZLI 5800, MLC 6613, and MBBA ((p-methoxybenzylidene)-p-butylaniline) and combinations thereof.

196. The method of Claim 129, wherein said substrate comprises a detection region comprising a recognition moiety.

197. The method of Claim 196, wherein said recognition moiety is selected from the group consisting of an peptide, polypeptide, protein, nucleic acid, carbohydrate, organic molecule, and inorganic molecule.

198. The method of Claim 197, wherein said protein is an antigen binding protein.

199. The method of Claim 196, wherein said substrate comprises a plurality of detection regions.

200. The method of Claim 199, wherein said plurality of detection regions display the same recognition moiety.

Figure 1

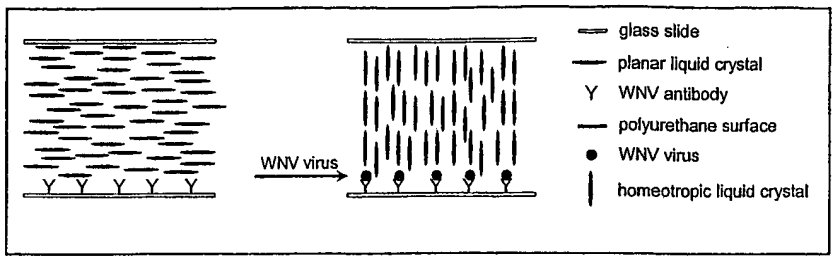
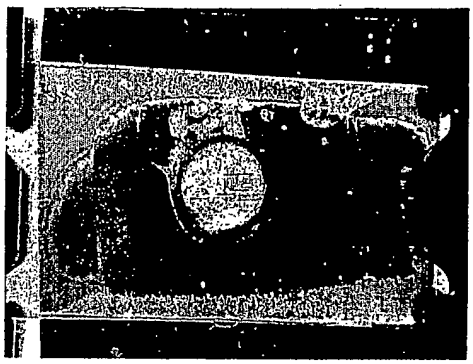
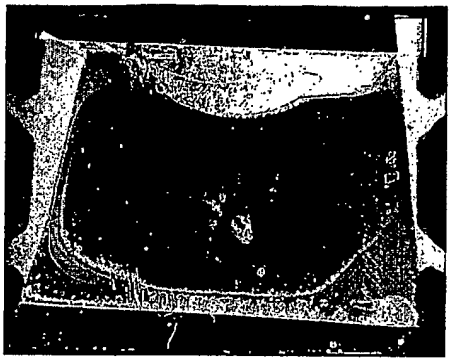

WNV virus
glass slide
planar liquid crystal
WNV antibody
polyurethane surface
WNV virus
homeotropic liquid crystal

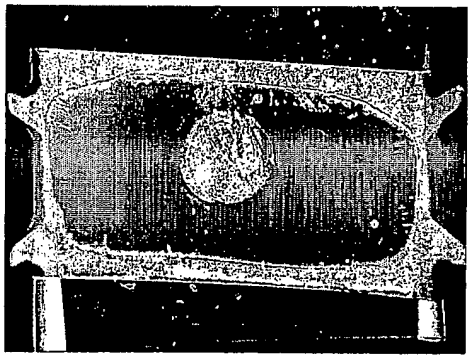
Figure 2

Anti-E Rabbit Polyclonal Antibodies - Rabbit Serum





+ Horse Serum - Horse Serum



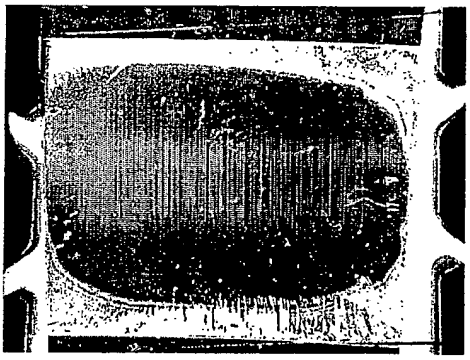


Figure 3

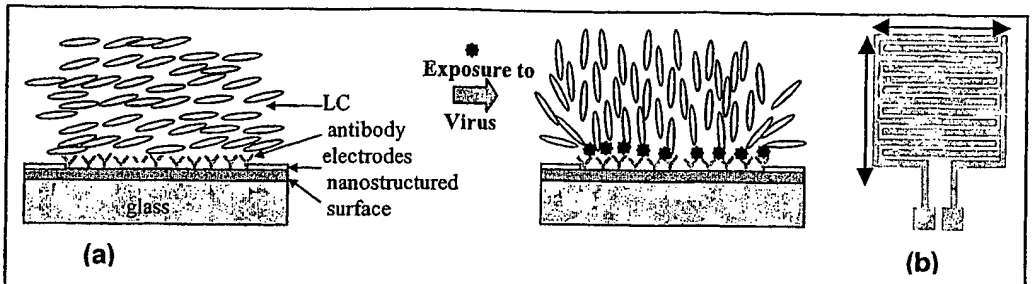


Figure 3. (a) The schematic cross-sectional view depicting the principle of detection of virus particle using LCs. The liquid crystal molecules supported on a nanostructured surface coated with antibody assume a planar orientation. Upon binding the virus particles on to the surface the molecules align perpendicular to the surface inducing a change in the capacitance between two electrodes. (b) Schematics of interdigitated electrodes. The size of the arrows is on the order of 500 μm.

Figure 4

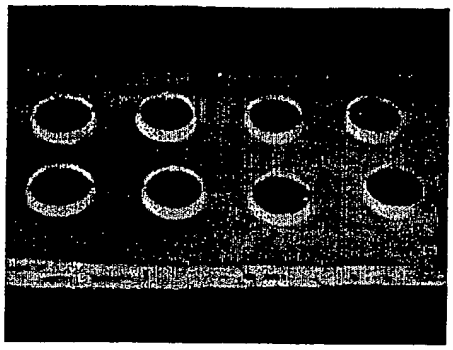


Figure 5

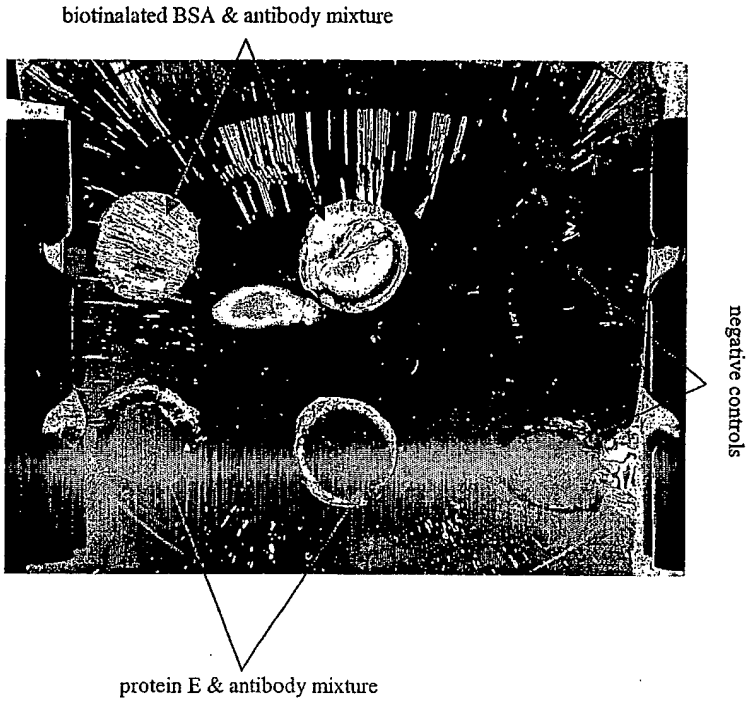

biotinalated BSA & antibody mixture
negative controls
protein E & antibody mixture

Figure 6

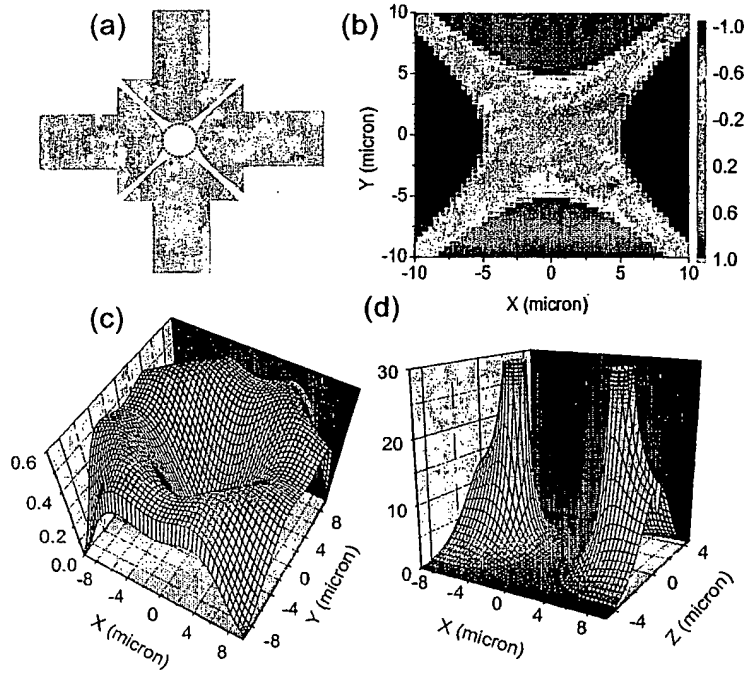


Figure 6. (a) Schematic of hyperbolic electrodes (b) the potential distribution in the plane of electrodes obtained by solving Laplace equation in 3D (c) the gradient of E^2 in a plane at $z = 4$ μm above the electrode plane and (d) on a $y = 0$ plane.

Figure 7

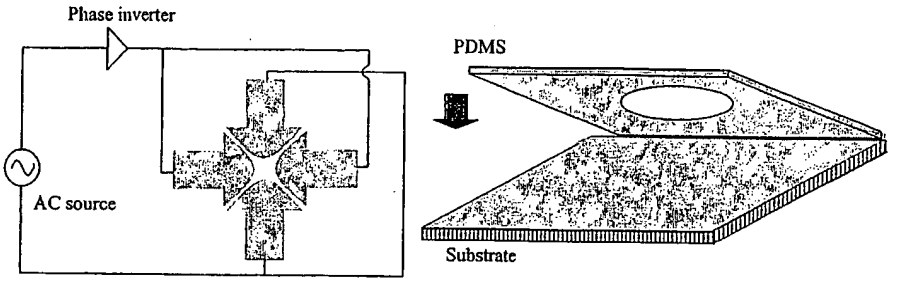


Figure 7. (a) A schematic of dielectrophoretic setup. (b) A 2 mm thick sheet of PDMS with a hole at the center confines the sample on the *active* region.

Figure 8

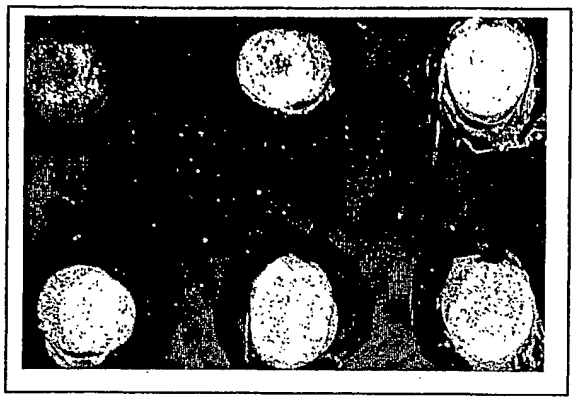


Figure 9

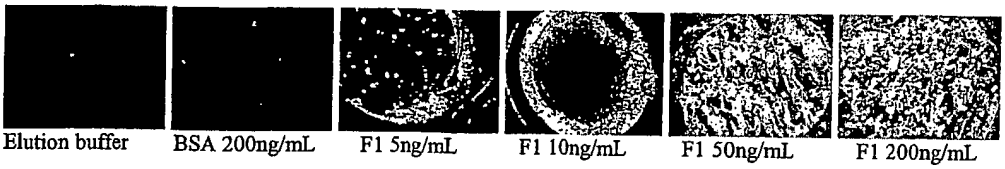


Figure 10

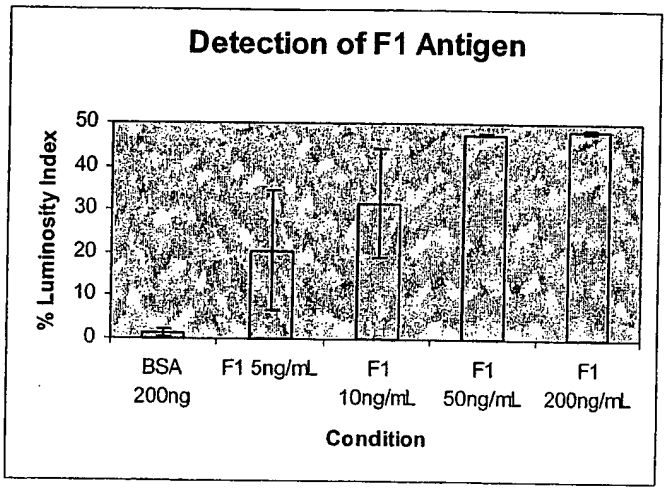


Figure 11

Figure 12

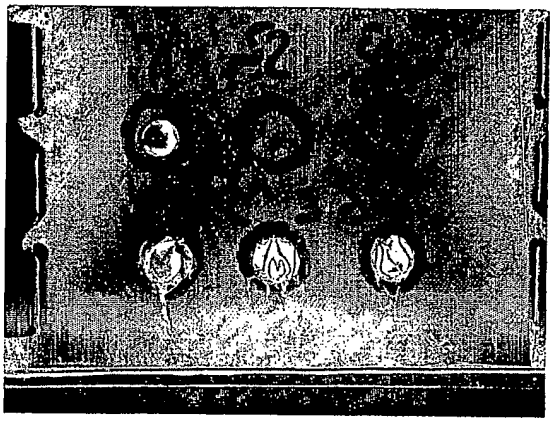
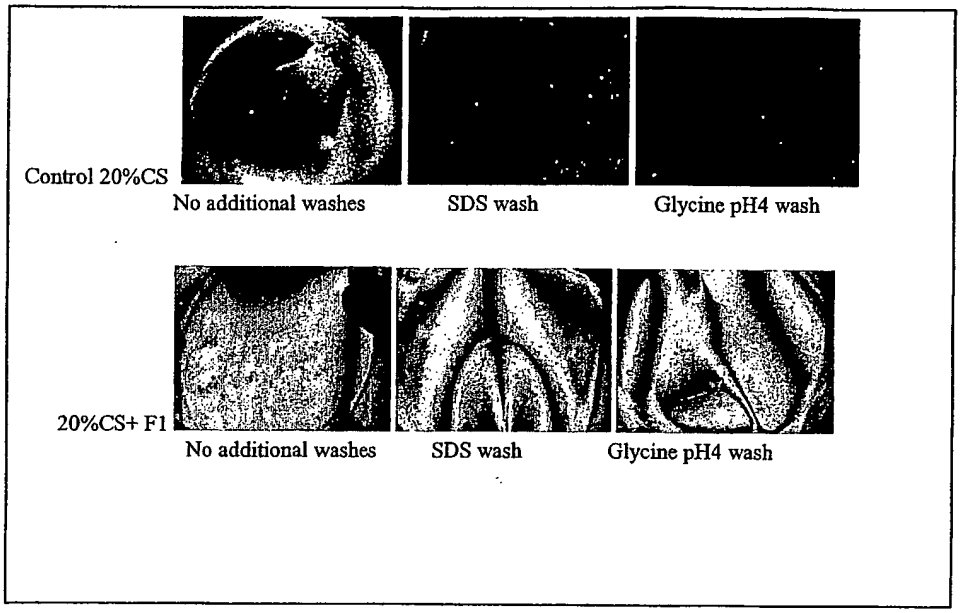


Figure 13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SDS wash
Glycine pH4 wash
20%CS+ F1
No additional washes
SDS wash
Glycine pH4 wash

Figure 14

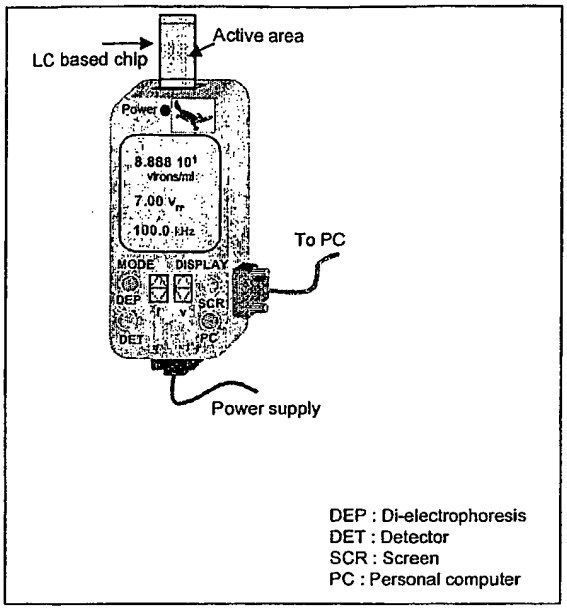
Active area
LC based chip
Power
8.888 10¹
virons/ml
7.00 Vpp
100.0 kHz
To PC
MODE
DISPLAY
DEP
SCR
DET
PC
Power supply
DEP : Di-electrophoresis
DET : Detector
SCR : Screen
PC : Personal computer

Figure 15

Anti-biotin antibody (100ug/ml) immobilized substrates after exposed to:

PBS buffer Biotin labeled liposome unlabeled liposome

Anti-biotin antibody (20ug/ml) immobilized substrates after exposed to:

PBS buffer Biotin labeled liposome unlabeled liposome